

**RESPONSE OF SQUASH PLANTS GROWN IN WINTER SEASON TO
SOME NATURAL EXTRACTS AND ANTIOXIDANTS**

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ABSTRACT

Two field experiments were conducted to study the effect of seed pre-sowing treatment with 100 & 200 ml/l of yeast or carrot extract and 250 & 500 ppm of ascorbic or citric acid on some growth aspects, leaf anatomy, fruiting and fruit quality of squash cv. Eskandarani grown under winter conditions during 2004 and 2005 seasons.

The results showed that, different applied treatments significantly increased all studied growth parameters as stem length and diameter, number of leaves/ plant, total leaf area/ plant and fresh and dry weights of both stems and leaves. Yet, significant reduction in the assimilation rate was existed with all applied treatments.

Besides, the two concentrations of each applied extract or antioxidant obviously increased photosynthetic pigments, NPK, sugars, total carbohydrates and crude protein concentrations in leaves of treated plants compared with those of untreated ones.

In addition, the obtained vigorous growth of squash with different applied treatments was accompanied by an obvious alteration in many anatomical features of leaves. Here, all applied treatments increased thickness of lamina and its comprising tissues as upper and lower epidermis, palisade and spongy tissues. Moreover, thickness of the main vein, dimensions of vascular bundle, thickness of both phloem and xylem tissues were also increased.

Furthermore, the applied extracts and antioxidant treatments also altered the sex ratio to be in favour of female flowers and earliness of fruit production. The highest early and total yields were obtained with 200 ml/l of yeast extract followed by 200 ml/l of carrot extract, 500 ppm of both ascorbic and citric acids, respectively. Meanwhile, chemical composition as minerals, sugars, carbohydrates, vitamin C, total soluble solids in squash fruits were also increased. Therefore, the present study strongly admit the use of such natural extracts and antioxidants as a pre-sowing treatments not only to increase earliness and total squash fruit production but also to avoid all cautions about inserting greenhouses in the agricultural system.

INTRODUCTION

Squash, *Cucurbita moschata* (Duchesne ex Lam.) Duchesne. ex Poir., is one of the important vegetables grown in Egypt. It is cultivated in Egypt all over the year, outdoor in summer and indoor either in greenhouses or in tunnels in winter. It is one of warm requiring vegetable crops and it does not tolerate either cooler or frosty weathers during germination and different stages of growth and development without indoor protection. Squash is injured when exposed to nonfreezing temperatures, i.e., below 12°C (Rab and Saltveit, 1996).

Imposing squash plants in the midst part of Egypt to frosty weather as well as to low temperature in the northern represent the main adverse factor for production of squash in winter and early summer plantings. Accordingly, there is a gap between production and consumption of squash fruits in the Egyptian market during these periods. To solve this problem, certain agricultural methods are always expensive.

Recently, great attention has been focused on the possibility to improve the ability of vegetables to tolerate cold stress. Among these treatments, seed cold hardening (Abd El-Dayem *et al.*, 2000) and the use of some chemicals and natural extracts of yeast and carrot (Fathy *et al.*, 2000).

On the other hand, it was demonstrated that all environmental stresses, cold, heat, salt, etc, either accelerate the formation of toxic oxygen free radicals (ROS) levels within plant tissues or impair the normal defense mechanisms that protect tissues from ROS toxic effect. Such stresses induce higher O₂ photo-reduction within chloroplasts or electron transport disturbance, and donation of electron to O₂ within mitochondria all led to generation of toxic ROS (Elstner and Osswald, 1994 and Mackersie *et al.*, 1996). Those ROS (H₂O₂, OH and O₂) damaged chloroplast, reduced carbohydrates synthesis and exportation and hastened oxygen senescence (Dicknson *et al.*, 1991), attacked cell membranes leading to their degradation and leakage of cell solutes, denaturation of protein and enzymes, damage of nucleic acids, degradation of chlorophyll and suppression of all metabolic processes, finally senescence and death of cells and tissues (Cakmak and Marschner, 1992).

Recently, group of substances known as antioxidants or oxygen free radical scavengers were exogenously applied to protect against adverse effects of environmental, oxidative stress such as citric acid, carotenoids, ascorbate, α -tocopherol, glutathion and vitamins (Anton and Basseim, 1998; Anton *et al.*, 1999; Arisha, 2000 and Fathy *et al.*, 2003).

The present approach has to identify the effect of some natural extracts and antioxidants by which squash plant may reverse their internal bio-mechanisms to be act in direction of cold tolerance. Therefore, improving growth and fruiting of squash under cold stress conditions.

Herein, its beneficial to reviewed about the expected roles and advantages of the assumed treatments, i.e., yeast and carrot extracts; ascorbic and citric acids.

Yeast extract suggested to participate a beneficial role during stress due to its cytokinin content (Barnett, *et al.*, 1990), to improve the formation of flower initiation due to its effect on carbohydrates accumulation (El-Desouky *et al.*, 1998). Also, it was reported about its stimulatory effects on cell division and enlargement, protein and nucleic acid synthesis as well as chlorophyll formation (Fathy and Faried, 1996). Add to its content of cryoprotective agent i.e., sugars, protein and amino acids and also several vitamins (Shady, 1978). Improving growth and fruiting of economical plants by yeast application was reported by El-Desouky *et al.*, (1998) and Wanas (2002).

Carrot extract, a rich natural source of carotenoides, suggested to be antioxidant material and used for enhancement of ABA bio-synthesis add to its content of sugars and antioxidant vitamins (A&C). Moreover, carotenoids known to be important precursor for ABA bio-synthesis via enzymatically degradation process (Bartels and Watson, 1978). Also, there is much evidence about the important role of endogenous ABA in induction of cold tolerance via controlling the expression of responsible genes and other associated favourable consequences (Frank, 1990)

As for, ascorbic and citric acids known to be important antioxidants due to their molecules auto (ox-redox.) properties act as cofactor for some specific antioxidant enzymes, i.e., dismutase, catalase and peroxidase, those catalyzed breakdown of the toxic (H_2O_2), (OH), (\dot{O}_2)radicals (Elad, 1992 and Aono *et al.*, 1993) . Some studies have been reported that ascorbic and citric acids had positive effects on plant growth and development due to its role in alleviating cold stress conditions (El-Lithy *et al.*, 2001 and Fathy *et al.*, 2003).

Therefore, the objective of the present study is to alleviate the adverse effects of low temperature and its probable accompanied oxidative stress on squash towards improving its growth and productivity during winter plantings by using some natural extracts (yeast and carrot extracts) and antioxidants (ascorbic and citric acids) as alternative possibility for using greenhouses production .

MATERIALS AND METHODS

Two field experiments were carried out during 2004 and 2005 winter seasons at the Experimental Farm of the Agricultural Botany Department, Faculty of Agriculture at Moshtohor, Benha University, Egypt. Seeds of the squash, *Cucurbita moschata* (Duchesne ex Lam.) Duchesne. ex Poir., cv. Eskandarani secured from the Egyptian Agriculture Res. Center, Ministry of Agric., A.R.E. Squash seeds were sown after being imbibed for 6 hours at room temperature ($20 \pm 2^\circ C$) in the two assigned concentrations of yeast or carrot extract (100 and 200 ml/l) and ascorbic or citric acid (250 and 500 ppm) as well as in distilled water as control treatment.

Preparation of extracts:**1- Yeast extract (YE):**

It was prepared by using a technique allowed yeast cells (pure dry yeast) to be grown and multiplied efficiently during conducive aerobic and nutritional conditions. To produce denovo beneficial bioconstituents, i.e., (carbohydrates, sugars, proteins, amino acid, fatty acids, hormones, etc.), hence allowed such constituents to release out of yeast cells in readily form by two cycles of freezing and thawing for disruption of yeast cells and releasing their content. Such technique for yeast preparation modified after Spencer *et al.* (1983).

Analysis of prepared yeast stock solution (Fathy, *et al.*, 2000) was: total protein (5.3%), total carbohydrates (4.7%), N (1.2%), P (0.13%), K (0.3%), Mg (0.013%), Ca (0.02%), Na (0.01%); micro-elements (ppm), Fe (0.13), Mn (0.07), Zn (0.04), Cu (0.04), B (0.016), Mo (0.0003), IAA (0.5 mg/ml) and GA (0.3 mg/ml). Yeast extract YE was used at two concentrations, i.e., 100 and 200 ml/l.

2- Carrot extract (CaE):

One Kg of fresh carrot roots, cleaned, rinsed and blended well, hence successive extractions were participated by different solvents, petroleum ether 100% (1 liter) and ethanol 50% (1 liter), respectively, each for 12 hours. Starring filtration and solvent volatilization were done, then volume of extract completed to liter by distilled water. Also, chemical analysis of dried carrot roots (Fathy *et al.*, 2000) was as follows: total carotenoids 12.80 mg/100g d.wt.), total sugars (278.50 mg/100g d.wt.) and vitamin C (12.46 mg/100g f.wt.). Carrot extract (CaE) was applied at two concentrations, i.e., 100 and 200 ml/l.

Hence, the experiment included 9 treatments i.e., the control (distilled water), 100 and 200 ml/l of each of yeast and carrot extracts and 250 and 500 ppm of each of ascorbic and citric acids. Soaked-seeds of each treatment in both seasons 2004 and 2005 at 4th of January were sown in open field in rows on one side of ridge 3.5 m length and 0.6m width at 0.4m apart with 3 ridges per experimental plot of 10.5m² area. The experiment was performed as a randomized complete block design in five replicates. Also, the normal agricultural practices of growing squash plants were followed up.

Sampling date and collecting data**I- Growth characters:**

Fifty days after sowing a random sample of five plants was chosen from each treatment. In each sample, length and diameter of the stem, number of leaves, total leaf area and the fresh & dry weights of both stem and leaves per plant were recorded. Diameter of stem was measured at the base of hypocotyl. While, leaf area was determined using the disk method as described by Derieux *et al.* (1973). Also, assimilation rate (A.R.) was calculated according to Wareing and Phillips (1981) using the following equation:

$$A.R = \frac{\text{Total leaf area (cm}^2\text{)} / \text{plant}}{\text{Total dry weight of leaves (g)/plant.}}$$

II- Photosynthetic pigments:

Chlorophyll a, b and carotenoids were colorimetrically determined in squash leaves at 50 days after sowing in both seasons according to the method described by Nornal, 1982

III- Anatomical study:-

According to the wide differences in the morphological characters of squash plants due to treatments in the first season a comparative anatomical studies on leaves of treated plants compared with those of the control were microscopically examined during the second season.

At 50 days after sowing, specimens (1cm²) were taken from the middle part of blade of the 4th apical leaf on the main stem. The specimens were killed and fixed for at least 48 hours in F.A.A. solution, washed in 50% ethyl alcohol, dehydrated in a series of ethyl alcohols (70, 90, 95 and 100%), infiltrated in xylene, embedded in paraffin wax of a melting point 60-63 °C (Sass, 1950), sectioned at 20 µ using a rotary microtome, double stained with fast green and safranin (Johanson, 1940), cleared in xylene and mounted in Canda balsam.

The prepared sections were microscopically examined. Counts and measurements (µ) were taken using a micrometer eye piece. Averages of readings from 4 slides/ treatment were calculated.

IV- Flowering and fruiting characters:

Five plants per each treatment were randomly chosen, labeled and the following data were recorded:

- a) Number of male and female flowers / plant were counted all over the season.
- b) The sex ratio was calculated as the rate of male/female flowers.
- c) Number and weight (g) of early formed fruits /plant (as the early four pickings).
- d) Early yield percentage /plant was calculated as a percentage of the total yield /plant.
- e) Number and weight (kg) of total fruits /plant.
- f) Relative total yield was calculated as a percentage of control yield.

V- Chemical constituents in the leaves and fruits:

Samples from squash leaves (50 days after sowing) and fresh marketable sized picked – fruits were taken to determine total carbohydrates (Dubois *et al.*, 1956), total and reducing sugars (Thomas and Dutcher, 1924), total nitrogen (Horneck and Miller, 1998), phosphorus (Sandell, 1950) and potassium (Horneck and Hanson, 1998). Also, crude protein was calculated according to A.O.A.C (1990) using the following equation:

$$\text{Crude protein} = \text{total nitrogen} \times 6.25$$

In addition, in fresh fruits, a hand refractometer and the method of A.O.A.C (1990) were used for the total soluble solids (TSS) and vitamin C determinations, respectively.

VI- Statistical analysis:

Data of growth, flowering and yield were subjected to statistical analysis according to Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

I- Growth characteristics:

Data in Table (1) revealed that different estimated growth parameters of stems (length, diameter, fresh and dry weights) and leaves (number, leaf area, fresh and dry weights) were significantly increased by application of all assigned treatments during both seasons compared with those of untreated plants. The most pronounced effect in this respect was shown with yeast extract (YE) followed by carrot extract (CaE), meanwhile ascorbic acid ranked the third and citric acid was the last one. Besides, the high concentration of all was more effective than the low one.

Table (1): Growth behaviour of squash plant as affected by some natural extracts and antioxidants applied as seed- soaking materials during 2004 & 2005 seasons.

Characters		Stem				Leaves				*A.R. cm ² /g
		Length (cm)	Diameter (cm)	Fresh weight (g)/plant	Dry weight (g)/plant	No./plant	Total leaf area/cm ² /plant	Fresh weight (g)/plant	Dry weight (g)/plant	
Treatments		Season 2004								
Control		10.40	1.10	5.26	0.39	9.60	767.67	24.94	3.78	203.09
Yeast	100ml/l	16.60	1.50	11.12	0.83	11.00	1137.84	40.10	6.52	174.52
extract	200ml/l	18.20	1.50	12.36	0.89	12.40	1615.55	60.46	9.32	173.34
Carrot	100ml/l	13.60	1.35	8.04	0.65	11.00	1034.27	36.45	5.86	176.50
extract	200ml/l	17.20	1.50	10.60	0.82	11.40	1528.82	54.02	8.74	174.92
Ascorbic	250ppm	13.20	1.35	7.98	0.66	10.60	928.44	30.12	4.98	186.43
acid	500ppm	15.60	1.45	9.76	0.73	11.40	953.56	34.36	5.38	177.24
Citric	250ppm	12.40	1.20	7.68	0.61	10.40	889.66	29.42	4.73	188.09
acid	500ppm	14.40	1.25	8.88	0.68	11.00	934.44	31.76	5.06	184.67
L.S.D	0.05	0.95	0.16	0.73	0.11	0.40	58.79	2.20	0.28	9.63
Season 2005										
Control		11.60	1.10	6.80	0.48	10.00	852.38	30.04	4.05	210.46
Yeast	100ml/l	17.60	1.50	11.62	0.91	11.80	1341.06	45.50	7.29	183.96
extract	200ml/l	21.80	1.62	13.16	1.04	13.00	1941.85	70.44	10.64	182.50
Carrot	100ml/l	14.20	1.40	8.88	0.79	12.20	1155.43	40.72	6.22	185.76
extract	200ml/l	19.10	1.60	13.20	0.98	12.80	1722.93	60.72	9.30	185.26
Ascorbic	250ppm	14.60	1.35	8.46	0.76	11.40	994.53	35.92	5.27	188.72
acid	500ppm	16.40	1.50	10.38	0.88	12.00	1118.94	40.18	6.08	184.04
Citric	250ppm	13.20	1.30	8.08	0.72	11.00	960.41	34.38	5.01	191.70
acid	500ppm	15.60	1.25	10.12	0.78	11.40	1075.90	35.86	5.69	189.09
L.S.D	0.05	1.22	0.21	0.81	0.16	0.51	74.11	2.86	0.34	9.23

- A.R. = Assimilation rate

Also, of interest to note that some of growth parameters in case of both yeast and carrot extracts specially at 200 ml/l reached more than two times of control values. Of these are stems and leaves dry weight and leaf area as well. Increment of leaf area is of great interest because that could be reflected upon the efficiency of photosynthesis by accumulation more assimilates and high rate of their translocation especially toward formed fruits.

As for, the calculated assimilation rate (proportion of leaf area (cm²) to leaves dry weight (g) / plant) exhibited its significant reduction with all applied treatments compared with that of untreated plants in both seasons. That means on one side that more amount of dry matter was produced from each unit of leaf area and on another side supports the above mentioned data about vigorous growth of squash as affected by different applied treatments.

The promotional effect of the natural yeast and carrot extracts on squash growth under cold stress is in agreement with the findings of El-Mogy *et al.* (1998) and Fathy *et al.*, (2000) on tomato plants. On the other hand, the positive effect of both ascorbic and citric acids is coincided with the findings of Arisha (2000), El-Lithy *et al.* (2001) and Hala *et al.* (2005) on other plants .

Regarding, the effect of soaking squash seeds in the assigned extracts and antioxidants under such cold conditions, it could be concluded that these treatments not only increased the ability of squash plants to withstand the low temperature of the winter surrounding conditions but also induced them to grow well under these adverse conditions . Also, the obtained results showed the superiority of yeast extracts in this concern than other treatments, i.e., carrot extract, ascorbic and citric acids in descending order.

For the advantageous effect of yeast extract could be due to its bioconstituents, i.e., carbohydrates, protein, GAs, IAA and vitamins as well as minerals content (see analysis of yeast extract). Besides, it might be due to its cytokinins content (Barnett *et al.*, 1990), its stimulatory effect on cell division and enlargement, protein and carbohydrates synthesis as well as chlorophyll formation (El-Desouky *et al.*, 1998 and Wanas, 2002).

Meanwhile, carrot extract treatment also assumed to be effective during cold stress mainly as a natural precursor of ABA. (carotenoids content) as well as sugars and antioxidant vitamins source. There is much evidence about the important role of ABA in induction of cold tolerance via its involvement in controlling gene expression and other associated favourable consequence (Frank, 1990). Carotenoids, the main constituents of carrot extract, known to be readily enzymatically degraded to ABA (Bartels and Watson, 1987). Beside to its content of available sugars and vitamins A (caroten) & C those known as anti-oxidant agents that delaying senescence of plant cells specially during stresses.

The beneficial effect of antioxidants i.e., ascorbic and citric acids on squash growth under cold conditions might be due to: (1) their action as cofactors for some specific enzymes, i.e., dismutase, catalase and peroxidase those catalyzed breakdown of the toxic (H₂O₂), (OH) & (O₂) radicals (Elad, 1992 and Aono *et al.*, 1993), (2) their stimulative effect on carotenoids synthensis (pro-VA

antioxidant) as a defensive mechanism against stress adverse effects (Table.2) and findings of Elad (1992); Aono (1993) and Hala *et al.*, (2005), (3) their enhancement of cell division and /or cell enlargement (Arrigoni, *et al.*, 1997) and /or (4) DNA replication (Noctor and Fayer, 1998) .

II- Photosynthetic pigments:-

Data in Table (2) indicate that application of yeast and carrot extracts each at 100 & 200 ml/l as well as ascorbic and citric acids at 250 & 500 ppm of each as seed - soaking treatments obviously increased photosynthetic pigments as chlorophyll a, b and carotenoids in leaves of treated plants more than those of untreated plants . Also, it is clear that each individual pigment and their sum were increased mostly in parallel to the concentrations used of each extract and antioxidant in both seasons.

Table (2): Photosynthetic pigments concentration in squash leaves as affected by some natural extracts and antioxidants during 2004& 2005 seasons.

Determinations Treatments		Season 2004				
		*Chl.			**Carot.	Chl.(a+b) + carot.
		a	b	a +b		
Control		0.52	0.33	0.85	0.46	1.31
Yeast	100ml/l	0.64	0.42	1.06	0.59	1.65
extract	200ml/l	0.74	0.54	1.28	0.69	1.97
Carrot	100ml/l	0.62	0.39	1.01	0.55	1.56
extract	200ml/l	0.67	0.44	1.11	0.61	1.72
Ascorbic	250ppm	0.58	0.40	0.98	0.54	1.52
acid	500ppm	0.66	0.46	1.12	0.60	1.72
Citric	250ppm	0.54	0.38	0.92	0.49	1.41
acid	500ppm	0.59	0.42	1.01	0.53	1.54
		Season 2005				
Control		0.49	0.32	0.81	0.44	1.25
Yeast	100ml/l	0.67	0.46	1.13	0.62	1.75
extract	200ml/l	0.79	0.55	1.34	0.71	2.05
Carrot	100ml/l	0.62	0.37	0.99	0.56	1.55
extract	200ml/l	0.69	0.45	1.14	0.63	1.77
Ascorbic	250ppm	0.61	0.41	1.02	0.56	1.58
acid	500ppm	0.67	0.48	1.15	0.61	1.76
Citric	250ppm	0.55	0.40	0.95	0.52	1.47
acid	500ppm	0.62	0.43	1.05	0.56	1.61

* Chl. = Chlorophyll **Carot. = Carotenoids.

In this respect, similar results about the stimulative effect of yeast and carrot extracts were reported by El-Desouky *et al.*, (1998) on squash and Wanas (2002), on broad bean. Besides, yeast treatment suggested to participate a beneficial role during vegetative growth through enhancement the chlorophyll formation and photosynthetic efficiency due to its content of cytokinins (Barnett *et al.*, 1990 and Fathy and Farid, 1996) . But in case of carrot extract, it was suggested to be used for enhancement of some hormones (GAs & ABA) biosynthesis beside its content of antioxidants vitamins A & C (Bartels and Watson, 1987) .

On the other hand, Hala *et al.*, (2005) reported similar results about the positive effect of antioxidants on photosynthetic pigments in broad bean plants. Increment of photosynthetic pigments in response to ascorbic and citric acids might be due to their role as antioxidants in protecting chloroplasts from oxidative damage by free radicals (Munne *et al.*, 2001).

III- Minerals and bioconstituents in squash leaves:

As shown in Table (3) yeast and carrot extracts as well as ascorbic and citric acids at their applied concentrations considerably increased N,P and K concentrations in leaves of treated plants during 2004 and 2005 seasons compared with those of untreated plants. Also, total sugars and carbohydrates as well as crude protein contents were positively responded to different applied treatments. The high concentration of each extract and antioxidant was more active than the low one. Moreover, the highest values were mostly obtained with yeast extract followed by carrot extract, ascorbic and citric acids, respectively.

Table (3): NPK and some bioconstituents concentrations in squash leaves as affected by some natural extracts and antioxidants applied as seed-soaking materials during 2004 & 2005 seasons.

Determination Treatments	Mg/g D.W.					Mg/g F. W.		
	N	P	K	Crude protein	Total *Carb.	**R. sugars	Non-R. sugars	Total sugars
Season 2004								
Control	24.83	2.36	23.60	155.19	513.82	6.33	2.82	9.12
Yeast 100ml/l	29.32	2.98	25.80	183.25	564.39	9.10	4.88	13.98
extract 200ml/l	34.16	3.76	28.52	213.50	633.42	10.63	6.92	17.55
Carrot 100ml/l	27.76	2.84	25.53	173.50	536.40	8.76	3.94	12.60
extract 200ml/l	31.94	3.42	27.78	199.63	591.50	10.12	5.52	15.64
Ascorbic 250ppm	28.54	2.79	25.68	178.38	572.35	8.10	4.64	12.74
acid 500ppm	30.18	3.37	27.38	188.63	611.47	10.06	6.70	16.67
Citric 250ppm	25.96	2.64	24.10	162.25	524.90	7.57	3.64	11.11
acid 500ppm	29.13	2.88	26.74	182.07	560.47	7.52	4.37	11.89
Season 2005								
Control	26.24	2.48	25.18	164.50	491.19	5.68	2.66	8.34
Yeast 100ml/l	30.14	3.20	27.66	188.38	584.32	9.60	5.23	14.83
extract 200ml/l	36.85	3.56	28.16	230.31	655.84	11.49	7.18	18.77
Carrot 100ml/l	28.90	2.91	26.98	180.63	543.13	9.02	4.12	13.14
extract 200ml/l	34.36	3.54	28.96	214.75	618.25	10.86	5.83	16.69
Ascorbic 250ppm	31.12	2.90	27.13	194.50	585.36	9.42	5.06	14.48
acid 500ppm	33.88	3.46	28.45	211.75	629.90	10.12	6.85	16.97
Citric 250ppm	28.15	2.78	26.69	175.94	563.18	8.43	3.83	12.26
acid 500ppm	30.64	3.18	27.34	191.50	592.16	9.52	4.52	14.04

* Carb. = Carbohydrates

** R. = Reducing

The obtained results are in harmony with those obtained by Fathy *et al.*, (2000) and Wanas (2002) for yeast and carrot extracts on tomato and broad bean, respectively. While, the positive effect of ascorbic and citric acids on minerals

and bioconstituents concentration is in agreement with the findings of Anton and Basseim (1998) and Anton *et al.*, (1999) on peanut and barley plants, respectively

Herein, increases of leaf area (Table, 1), and photosynthetic pigments (Table, 2) as well as increment of the dry matter accumulation in leaves indicate the positive and stimulatory effects of those natural extracts and antioxidants upon the efficiency of photosynthesis process, hence more photosynthates being created as well as enhancement of mineral translocation from roots to leaves.

Carbohydrates and sugars link to the case of cold tolerance (Frank, 1990) via their roles as cellular cryoprotective or osmoprotectors agent (Hockaka and Somero, 1973), they protected proteins and enzymes against denaturations induced by cold stress, as well as basic substrate for ATP synthesis. Phosphorus uptake and content depressed by low temperature (Table, 3&7) known to be taken as indicator for energy status, so its content directly associated with cold tolerability or sensitivity.

IV- Leaf blade anatomy:

As shown in Table (4) and Figs. (1&2) nearly different measured or counted anatomical features of squash leaf blade were positively affected with different applied treatments. Since, thickness of the main vein was increased over the control value by 10.88 & 22.51%; 14.09 & 24.91%; 9.23 & 14.39% and 7.38 & 13.56% with YE at 100 & 200 ml/l; CaE at 100 & 200 ml/l; ascorbic acid at 250 & 500 ppm and citric acid at 250 & 500 ppm, respectively. Also, it could be noticed that the highest increase value of this trait existed with CaE at 200 ml/l followed by YE at 200 ml/l.

As for the length of main vascular bundle, it was also increased with different applied treatments. Increase value reached its highest value (585.50 μ) with YE at 200 ml/l that represent 130.40% when compared with the control (100%). Also, the width of this main vascular bundle was increased with all applied treatments to reach its maximum with the same treatment as in case of its length (i.e., YE at 200 ml/l). In addition, thickness of both uppermost and lowermost phloem tissues were increased with the assigned treatments. Here, it is evident that YE at 200 ml/l gave the highest values of these phloem traits (Fig., 1).

On the other hand, thickness of xylem tissue was also increased with different applied treatments to reach its maximum with CaE at 200 ml/l that reached about 19% of increase. As for the number of xylem vessels in the main vein bundle, it was also increased with all applied treatments especially with the two concentrations applied of yeast and carrot extracts.

With regard to lamina thickness, as shown in Table (4) and Fig. (2), it was also increased with different applied treatments to reach its maximum with YE at 200 ml/l (62% of increase) followed by YE at 100 ml/l (33% of increase). As regard to upper and lower epidermis also nearly increased in all treatments.

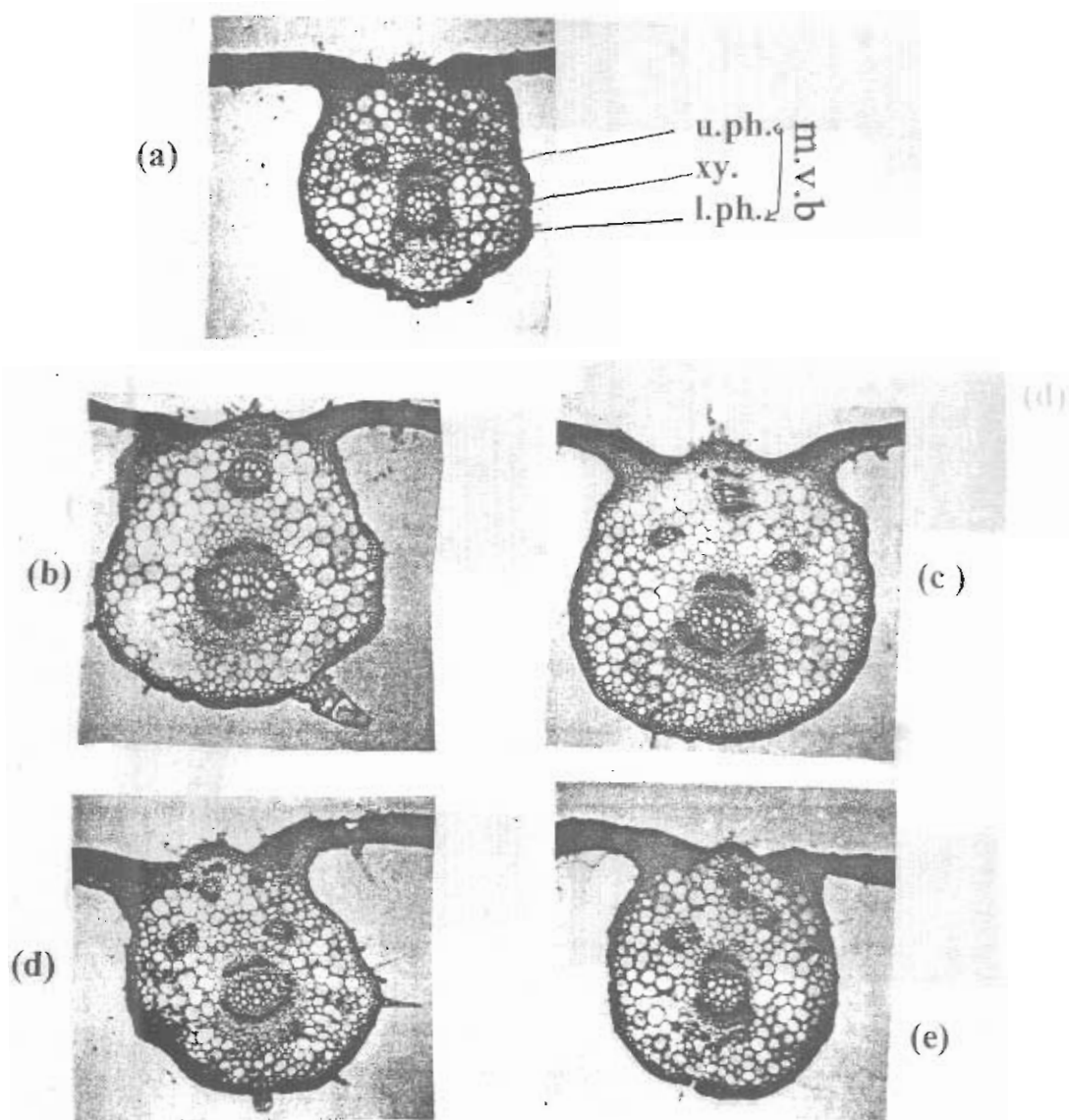


Fig. (1): Transverse sections through the main vein of 4th apical leaf blade on the main stem of squash as affected by natural yeast and carrot extracts as well as ascorbic and citric acids applied as seed-soaking treatments (X40).

a-Control

b. Yeast extract at 200 ml/l

c-Carrot extract at 200 ml/l

d- Ascorbic acid at 500 ppm

e- Citric acid at 500 ppm

Abb: u. ph. = uppermost phloem tissue, xy. = xylem tissue, l.ph. = lowermost phloem tissue and m.v.b. = main vascular bundle.

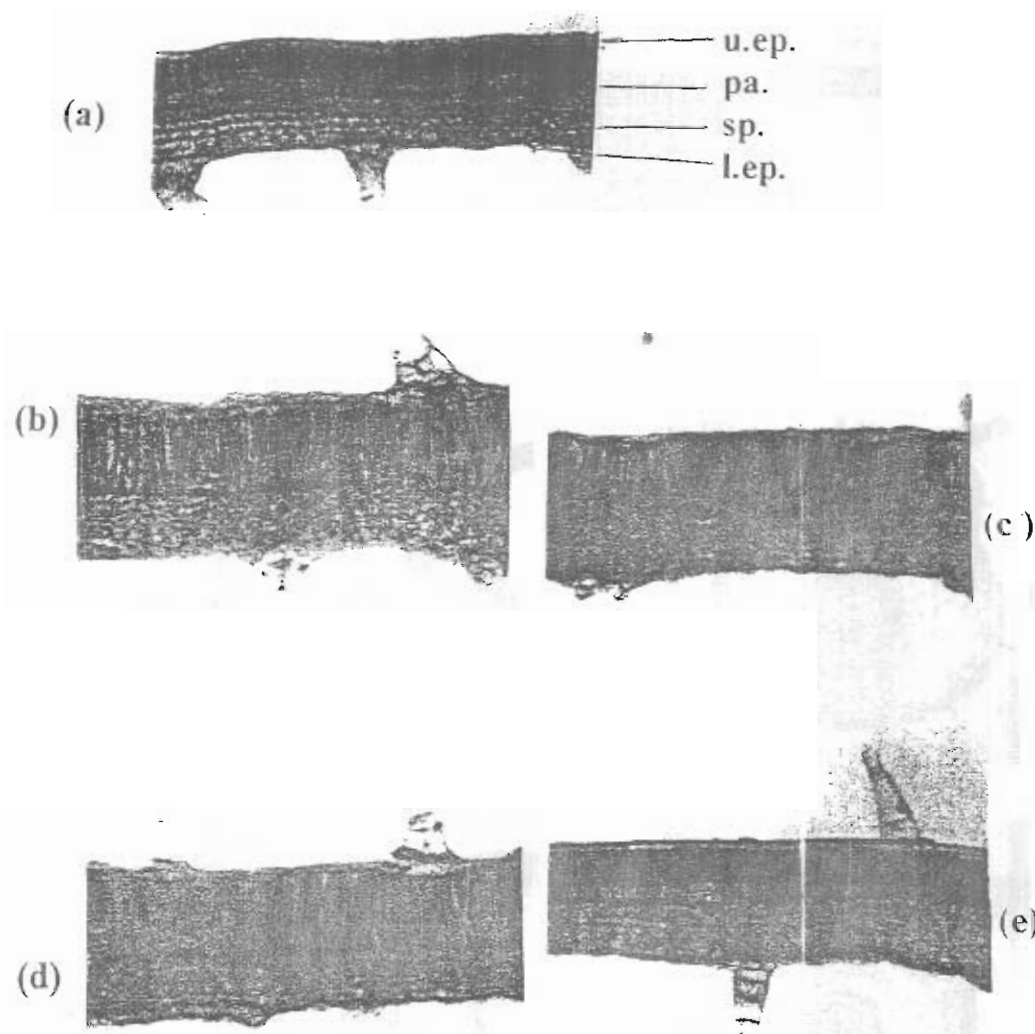


Fig. (2): Transverse sections through the lamina of 4th apical leaf blade on the main stem of squash as affected by natural yeast and carrot extracts as well as ascorbic and citric acids applied as seed-soaking treatments (X100).

a-Control

b- Yeast extract at 200 ml/l

c- Carrot extract at 200 ml/l

d- Ascorbic acid at 500 ppm

e- Citric acid at 500 ppm

Abb u.ep.= upper epidermis, l.ep.= lower epidermis, pa.=palisade tissue and sp.= spongy tissue

The only exception was that of ascorbic acid of 250 ppm, since it didn't affect the thickness of lower epidermis.

With regard to the thickness of each of palisade and spongy tissues, different applied treatments obviously increased these traits. Also, the highest increase was obtained with YE at the two applied concentrations that gave increases of 38.59% for palisade tissue and 48.82% for spongy tissue with YE at 100 and 200 ml/l, respectively.

Table (4): Anatomy of squash leaf blade as affected by some natural extracts and antioxidants applied as seed-soaking materials.

Measurements (μ) & count		Thick. of main vein	Length of main vascular bundle	Width of main vascular bundle	Thick. of uppermost phloem tissue	Thick. of lowermost phloem tissue	Thick. of xylem tissue	No. of xylem vessels/ main bundle	Thick. of lamina	Thick. upper epidermis	Thick. of lower epidermis	Thick. of palisade tissue	Thick. of spongy tissue	
Treatments														
Yeast extract	Control *	X	1462.60	449.10	364.50	77.60	70.20	301.20	27.00	143.35	13.95	11.70	67.30	50.40
		X	1621.80	562.50	495.90	118.80	91.90	351.80	33.75	191.00	17.00	13.50	92.70	73.80
		%	110.88	125.25	136.05	153.90	130.91	116.80	125.00	133.24	121.86	115.38	137.74	146.43
	200ml/l	X	1791.90	585.45	542.70	131.40	108.60	346.45	35.25	232.10	18.90	15.30	107.10	91.80
		X	1791.90	585.45	542.70	131.40	108.60	346.45	35.25	232.10	18.90	15.30	107.10	91.80
		%	122.51	130.36	148.89	169.33	154.70	115.02	130.56	161.91	135.48	130.77	159.14	182.14
Carrot extract	100 ml / l	X	1668.70	495.90	445.90	83.70	92.90	319.60	33.25	173.70	16.20	13.50	78.30	65.70
		X	1668.70	495.90	445.90	83.70	92.90	319.60	33.25	173.70	16.20	13.50	78.30	65.70
		%	114.09	110.42	122.33	107.86	132.34	106.11	123.15	121.17	116.13	115.38	116.34	130.36
	200ml / l	X	1826.90	549.45	488.70	99.45	90.90	359.10	32.50	186.20	17.10	14.40	85.50	70.20
		X	1826.90	549.45	488.70	99.45	90.90	359.10	32.50	186.20	17.10	14.40	85.50	70.20
		%	124.91	122.34	134.07	128.16	129.49	119.22	120.37	129.89	122.58	123.08	127.04	139.29
Ascorbic acid	250ppm	X	1597.60	493.20	355.50	89.10	75.60	328.50	31.50	169.20	14.40	11.70	85.50	57.60
		X	1597.60	493.20	355.50	89.10	75.60	328.50	31.50	169.20	14.40	11.70	85.50	57.60
		%	109.23	109.82	92.04	114.82	107.69	109.06	116.67	118.03	103.23	100.00	127.04	114.29
	500 ppm	X	1673.10	487.20	369.45	85.50	72.00	321.20	29.25	188.80	15.30	12.60	98.10	63.90
		X	1673.10	487.20	369.45	85.50	72.00	321.20	29.25	188.80	15.30	12.60	98.10	63.90
		%	114.39	108.48	101.36	110.18	102.56	106.64	108.33	131.71	109.68	107.69	145.77	126.79
Citric acid	250ppm	X	1570.60	515.70	409.60	95.40	77.40	342.90	32.75	160.20	15.30	12.60	72.90	59.40
		X	1570.60	515.70	409.60	95.40	77.40	342.90	32.75	160.20	15.30	12.60	72.90	59.40
		%	107.38	114.83	112.37	122.44	110.26	113.84	121.30	111.75	109.68	107.69	108.32	117.86
	500ppm	X	1660.90	465.80	528.30	90.00	72.90	303.20	33.50	173.70	16.20	14.40	81.00	62.10
		X	1660.90	465.80	528.30	90.00	72.90	303.20	33.50	173.70	16.20	14.40	81.00	62.10
		%	113.56	103.72	144.94	115.98	103.85	100.66	124.7	121.17	116.13	123.08	120.36	123.21

* Control values are considered as 100% .

In general, the alteration of different traits of leaf anatomy with the all applied treatments is being of great interest. Because these alterations included an

increase in each of the thickness of photosynthates creator i.e. lamina tissue and the thickness of their passage (phloem tissue) as well as the thickness of different raw materials passage (absorbed by roots); i.e. xylem tissue thickness as well. That means that these treatments especially of yeast extract improved translocation and caused more raw materials to be absorbed by roots and reached to leaves as well as more photosynthates to be allocated and partitioned to other plant parts. In this respect, Atawia and El-Desouky (1997) and Wanas (2002) have been confirmed that the significant increase of yield in economical plants is mainly due to the increase of transverse sectional area of both xylem and phloem tissues.

Therefore, treatments applied in the present study especially those of natural extracts (i.e. yeast and carrot extracts) are being of great interest. Since, these treatments are being of economic value not only considering significant increase of obtained yield but also keeping good marketing, diet and taste characteristics as mentioned later.

V- Reproductive growth:

a- Sex expression:

As illustrated in Table (5) different applied seed - soaking treatments tended to affect male and female flower numbers and that was markedly for summer squash cv. Eskandarani during 2004 and 2005 winter seasons. Since, significant reduction in the male flowers number and increase in female ones were existed in parallel to the applied concentration of each extract and antioxidant. Also, yeast extract was the most effective treatment in this respect compared with another extract and the two antioxidants as well.

Table (5): Flowering characteristics of squash plant as affected by some natural extracts and antioxidants applied as seed – soaking materials during 2004 & 2005 seasons.

Characters Treatments		Season 2004			Season 2005		
		No. of flowers/plant		Male/female ratio	No. of flowers/plant		Male/female ratio
		Male	Female		Male	Female	
Control		38.40	16.60	2.31	40.60	18.20	2.23
Yeast extract	100ml/l	31.60	23.20	1.36	33.20	25.00	1.33
	200ml/l	30.40	26.20	1.16	31.80	27.80	1.14
Carrot extract	100ml/l	32.20	22.40	1.44	33.80	24.20	1.40
	200ml/l	30.80	24.80	1.33	31.60	26.40	1.20
Ascorbic acid	250ppm	32.80	21.80	1.50	35.60	23.40	1.53
	500ppm	31.40	23.60	1.33	33.40	25.80	1.29
Citric acid	250ppm	33.20	19.80	1.68	35.80	22.00	1.63
	500ppm	32.60	21.60	1.51	34.40	24.20	1.42
LSD	0.05	3.08	2.33	0.28	3.56	2.67	0.16
	0.01	4.15	3.14	0.38	4.80	3.60	0.22

In addition, by virtue of the reduction of the male flowers number and increasing the female ones, the male / female ratio was dominantly showed its high significant reduction with different applied extracts and antioxidants treatments.

Concerning these data, it could be concluded that, under cold conditions, the production of female flowers in Eskandarani squash cultivar was favoured, since different extracts and antioxidants treatments allowed seedling to achieve vigorous growth during this period of cultivation more than untreated plants. Moreover, those treatments encouraged the carbohydrates formation (Table, 3) . In this respect, Ne Smith *et al.*, (1994), reported that in squash, low temperature may inhibit the development of male flowers after differentiation leading to precocious female flowers. In addition, Wien (1997) concluded that conditions which enhance the building up of carbohydrates tend to favour female flowers expression, whereas factors reduce carbohydrates build-up, such as temperatures, also increase the tendency for male flowers production in the cucurbit vegetables.

Early and total fruit yields:-

Data in Table (6) indicate that different extracts and antioxidants applied as seed - soaking treatments increased early fruits number and yield / plant as well as early yield percentage to reach the two levels of significance during the two seasons under study. With regard to total fruits number and yield / plant, the calculated relative total yield as well as mean weight / fruit and percentage of dry matter / fruit were also increased to reach their maximum values with different applied treatments compared with the control treatment. Again, increases also, were mostly in parallel to the concentration used of each extract and antioxidant. Also, the highest values were obtained with yeast extract followed by carrot extract then ascorbic and citric acids, respectively.

Regarding the earliness of squash fruiting and increasing early fruits yield in response to different applied treatments, it could be attributed to the increasing of total leaf area and dry matter accumulation (Table, 1), photosynthetic pigments (Table, 2) and assimilates supply (Tables, 3 & 7) as well as enhancing leaf anatomy (Table, 4 and Figs., 1 & 2) under low temperature that favour femaleness and hence enhancement of fruits growth rates. In this respect, Marcelis (1993) reported that the higher assimilate levels and carbohydrates supply resulted in increased number and size of fruit cells.

c- Minerals and bioconstituents in the fruits:-

Data in Table (7) clearly show that during both seasons yeast and carrot extracts each at 100 & 200 ml/l as well as ascorbic and citric acids at 250 and 500 ppm applied as seed - soaking treatments markedly increased minerals (N,P, & K), crude protein, total sugars, carbohydrates and vitamin C concentrations as well as total soluble solids (TSS) in the marketable size squash fruits compared with the control .

The increase in total carbohydrates concentrations in the fruits could be indicated by improvement the squash growth concerning efficient photosynthesis

and improvement the translocation of their products as well as N, P and K to the ultimate fruits as affected by the applied pre-sowing treatments. Thereby, the obtained squash fruits under these treatments were of good quality.

In general, it could be concluded that soaking squash seeds in the assigned treatments i.e., yeast or carrot extract at 100 & 200 ml/l and ascorbic or citric acid at 250 & 500 ppm led to vigorous growth of squash seedlings and altered the gender of the formed flowers to be in favour of female ones. Besides, the soaking treatments also caused earliness of fruits production and increased their capacity under low temperature. That is reflected upon the ultimate fruits yield to reach highest values compared with the control as well as improved fruit quality. All of these advantages– favour the applied extracts and antioxidants treatments to be recommended as an important and effective agricultural practice in squash cultivation.

Table (6): Early and total yields of squash plant as affected by some natural extracts and antioxidants applied as seed – soaking materials during 2004 & 2005 seasons.

Characters Treatments		Early fruits No./plant	Early yield (g)/plant	Early yield (%)	Total fruits No./plant	Total yield (kg)/plant	Relative total yield (%)	Mean weight (g)/fruit	% of dry matter(g)/fruit
Season 2004									
Control		1.60	102.82	10.06	9.80	0.97	100.00	98.54	7.08
Yeast	100ml/l	2.80	185.70	13.36	13.20	1.39	143.30	104.94	7.88
Extract	200ml/l	3.60	229.50	14.08	15.40	1.63	168.04	105.05	8.52
Carrot	100ml/l	2.40	164.67	12.02	12.80	1.37	141.12	103.92	7.84
Extract	200ml/l	3.20	215.29	13.98	14.60	1.54	158.76	105.37	8.26
Ascorbic	250ppm	2.20	163.67	11.86	13.20	1.38	142.27	104.46	7.50
Acid	500ppm	3.00	201.68	13.72	14.00	1.47	151.15	105.03	8.08
Citric	250ppm	2.60	153.62	11.55	12.80	1.33	137.11	103.97	7.36
Acid	500ppm	3.20	193.10	12.96	14.40	1.49	153.61	103.36	7.78
LSD	0.05	0.52	7.87	0.56	1.66	0.21	-	3.67	0.13
	0.01	0.70	10.61	0.75	2.28	0.28	-	4.95	0.18
Season 2005									
Control		1.80	114.14	10.87	11.00	1.05	100.00	95.83	7.31
Yeast	100ml/l	3.40	211.35	14.09	14.60	1.50	142.86	102.76	8.02
Extract	200ml/l	4.00	251.47	14.88	16.20	1.69	160.95	104.19	8.43
Carrot	100ml/l	3.00	186.34	12.94	14.20	1.44	137.14	101.32	7.91
Extract	200ml/l	3.40	222.75	13.75	15.60	1.62	154.29	104.05	8.19
Ascorbic	250ppm	2.80	172.03	12.03	14.00	1.43	136.19	102.14	7.82
Acid	500ppm	3.00	206.06	12.96	15.40	1.59	151.14	102.98	8.19
Citric	250ppm	2.80	167.69	12.33	13.40	1.36	129.52	101.18	7.6
Acid	500ppm	3.00	212.51	13.45	15.20	1.58	150.48	103.74	7.83
LSD	0.05	0.63	6.81	0.84	1.19	0.26	-	3.12	0.18
	0.01	0.85	9.18	1.13	1.60	0.35	-	4.21	0.24

Table (7): NPK and some bioconstituents in squash fruits as affected by some natural extracts and antioxidants applied as seed - soaking materials during 2004 & 2005 seasons .

Determinations		Mg/g dry weight					Mg/g fresh weight			Vitamin C mg/100g fresh weight	TSS (%)
		N	P	K	Crude protein	Total **Carb.	Reducing sugars	Non-reducing sugars	Total sugars		
Treatments		Season 2004									
Control		20.43	2.85	22.60	127.69	669.54	12.62	5.08	17.70	8.94	3.65
Yeast	100ml/l	24.10	3.63	25.38	150.63	731.08	15.98	7.51	23.49	10.92	4.12
extract	200ml/l	26.18	4.12	26.18	163.63	742.86	21.19	8.49	29.68	11.86	4.45
Carrot	100ml/l	23.36	3.54	24.75	146.00	705.38	17.38	8.03	25.41	11.06	4.31
extract	200ml/l	25.24	3.95	26.26	157.75	757.43	23.75	9.33	33.08	13.27	4.76
Ascorbic	250ppm	22.53	3.26	24.08	140.81	700.97	16.68	6.52	23.20	13.24	3.95
acid	500ppm	25.15	3.58	24.82	157.19	728.28	19.25	7.39	26.64	14.60	4.25
Citric	250ppm	21.82	3.10	23.95	135.19	695.13	15.32	5.86	21.18	10.08	3.87
acid	500ppm	22.64	3.67	24.65	141.50	716.53	17.91	6.83	24.74	10.84	4.13
Season 2005											
Control		21.08	3.20	23.10	131.75	692.84	14.33	6.87	21.10	9.26	3.76
Yeast	100ml/l	24.36	3.57	25.85	152.25	739.44	18.65	9.16	27.81	11.76	4.15
extract	200ml/l	25.22	3.88	27.25	157.63	745.22	23.63	9.26	32.89	13.22	4.58
Carrot	100ml/l	23.64	3.73	25.03	147.75	732.16	19.52	9.23	28.75	11.83	4.40
extract	200ml/l	24.76	3.90	26.98	154.75	767.57	25.32	10.12	35.44	14.04	4.77
Ascorbic	250ppm	23.06	3.65	24.63	144.13	724.35	17.08	8.13	25.21	13.98	3.85
acid	500ppm	25.10	3.84	25.93	156.88	741.80	20.87	9.06	29.93	15.88	4.13
Citric	250ppm	22.35	3.47	24.49	139.69	713.67	16.87	7.26	24.13	11.17	3.93
acid	500ppm	23.45	3.72	25.77	146.56	731.64	18.08	8.13	26.21	12.09	4.10

* TSS = Total soluble solids

** Carb. = Carbohydrates

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استجابة نباتات الكوسة النامية في الموسم الشتوى لبعض المستخلصات الطبيعية ومضادات الأكسدة

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قسم النبات الزراعى – كلية الزراعة بمشتهر – جامعة بنها – مصر

أجريت تجربتين حقليتين لدراسة تأثير معاملة البذور قبل الزراعة بتركيزى ١٠٠ و ٢٠٠ مل/لتر من مستخلص الخميرة أو مستخلص الجزر وكذلك تركيزى ٢٥٠ و ٥٠٠ جزء فى المليون من حمض الأسكوربيك أو حمض الستريك على بعض خصائص النمو وتثريح الورقة ومحصول الثمار وكذلك جودتها لنباتات الكوسة صنف إسكندرانى النامية فى الموسم الشتوى خلال عامى ٢٠٠٤ و ٢٠٠٥م. أظهرت النتائج المتحصل عليها أن جميع المعاملات المستخدمة قد أدت إلى زيادة عالية المعنوية فى كل قياسات النمو المدروسة وهى طول وقطر الساق – عدد الأوراق المتكونة/نبات – مساحة الأوراق الكلية/نبات وكذلك الأوزان الطازجة والجافة لكل من السوق والأوراق. ومع ذلك حدث نقص معنوى فى معدل التمثيل مع كل المعاملات المستخدمة.

بجانب ذلك فإن كلا التركيزين المستخدمين من كل مستخلص أو مضاد أكسدة سبب زيادة واضحة في صبغات التمثيل الضوئي وكذلك محتوى الأوراق من عناصر النيتروجين ، الفوسفور ، البوتاسيوم وبعض المكونات الحيوية مثل السكريات والكربوهيدرات الكلية مقارنة بنظائرها في النباتات غير المعاملة .

بالإضافة إلى ذلك ، فإن النمو القوي لنباتات الكوسة نتيجة لتأثيرها بالمعاملات المستخدمة كان مصحوبا بتغيرات واضحة في بعض الصفات التشريحية خاصة للأوراق حيث أدت جميع المعاملات إلى زيادة سمك النصل وأنسجته المختلفة وهي البشرة العليا والبشرة السفلى ، النسيج العمادي والنسيج الإسفنجي . وعلاوة على ذلك فقد حدثت أيضا زيادة في سمك العرق الرئيسي وأبعاد الحزمة الوعائية الرئيسية وسمك نسيج الخشب واللحاء مع كل المعاملات المستخدمة مقارنة بنباتات الكنترول .

فضلا عن ذلك ، أدت معاملات المستخلصات ومضادات الأكسدة المستخدمة إلى تغيير النسبة الجنسية لتكون في اتجاه الأزهار المؤنثة (أي زيادة نسبة الأنوثة) وكذلك تكبير في إنتاج الثمار. وقد كانت أعلى زيادة في المحصول المبكر وأيضا المحصول النهائي مع التركيز العالي المستخدم من كل من مستخلص الخميرة يليه مستخلص الجزر ثم حمض الاسكوربيك والستريك على التوالي . وعلاوة على ذلك أظهرت المعاملات المستخدمة أيضا زيادة في محتويات الثمار من العناصر المعدنية ، السكريات ، الكربوهيدرات ، فيتامين ج وكذلك الجوامد الصلبة الكلية .

وبناء على ذلك ، فإن الدراسة الحالية تؤيد بقوة استخدام مثل هذه المستخلصات الطبيعية ومضادات الأكسدة كمعاملات تقع للبذور قبل الزراعة ليس فقط من أجل التذكير في المحصول وزيادة المحصول النهائي ولكن أيضا من أجل تجنب كل التحذيرات المتعلقة بإدراج الصوب في النظام الزراعي.