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UTILIZATION OF SOME NATURAL PLANT EXTRACTS (OF GARLIC & YEAST) AS SEED-SOAKED MATERIALS TO SQUASH (*Cucurbita pepo* L.) II- EFFECT ON THE HISTOLOGICAL FEATURES AND THE ENDOGENOUS HORMONES

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ABSTRACT

In pot experiments, pre-soaked squash seeds in 50, 250 and 500 ml/L either of natural garlic or yeast extracts were sown during 1997 and 1998 seasons.

At 40 days after sowing in 1998 season, the histological features were studied in design specimens of roots, stems and leaves and the endogenous phytohormones levels in leaves as well.

Both the natural garlic and yeast extracts with different applied concentrations led to increase different aspects of roots, stems and leaves anatomy. In this respect, diameter of the whole root section was more than the control with 65.58, 59.76 & 52.17% for garlic extract and 30.01, 42.14 & 66.15 for yeast extract by 50, 250 & 500 ml/L of each extract respectively. Also, the thickness of cambial region in the root was increased more than the control with 60.61, 54.55 & 45.45% for garlic extract and 31.82, 42.42 & 96.97 for yeast extract by 50, 250 & 500 ml/L of each extract, respectively. However, the number of vascular bundles in roots or stems was not changed.

Moreover, different applied concentrations of garlic or yeast extracts significantly increased the levels of endogenous auxins, gibberellins and cytokinins in leaves of treated plants.

INTRODUCTION

Nowadays, many of the agricultural chemical companies maintain their own screening facilities for testing new natural compounds for biological activities.

In this respect, many natural substances found in the environment are well-known as regulators of the growth and development of higher plants.

These hormonal substances originate from diverse sources. Bacteria and fungi from the soil rhizosphere or root surfaces, as well as bacteria on the surface of leaves, are capable of synthesizing and releasing hormones which affect the growth of the closely associated higher plants (Noggle & Fritz, 1992). Hormones can even be leached from leaves or released from roots of higher plants, and thereby alter the growth of other individuals in the plant community.

Some chemicals presently used in commercial agriculture and horticulture are structurally related to endogenous plant growth substances. In this respect, Nickell (1981) has listed some possibilities along with several of the chemicals now being used. However, many biologically active compounds bear little resemblance to natural substances. Since, in the absence of a molecular basis for growth substance activity, it is necessary simply to apply various organic molecules to plants and observe the results. Hence, biological screening procedures can be developed for selecting compounds for growth stimulation, yield enhancement and other aspects of plant development (Noggle & Fritz, 1992).

Therefore, according to the significant increases in both growth and fruit yield of squash that obtained in the first part of this study (El-Desouky *et al.*, 1998); it is reasonable to prolong this study to investigate different effects of garlic and yeast as natural extracts on the histological features and the endogenous phytohormones level in squash plants grown from treated seeds.

MATERIALS AND METHODS

Two pot experiments were conducted at the Agricultural experimental station of Agricultural Botany Department, Faculty of Agriculture at Moshtohor, Zagazig University, Egypt. Seeds of squash (*Cucurbita pepo* L.) variety "Eskandarani" were soaked in different extracts concentrations for four hours. Then were sown (in fertile garden soil 10 kg/pot) on 1st of March at 1997 and 1998 seasons.

Preparation of natural garlic and yeast extracts as well as the agricultural practices were made as previously mentioned in the previous study (El-Desouky *et al.*, 1998). Also, the endogenous hormones were determined in the two extracts directly before their using for soaking squash seeds (Fig. 6).

Three concentrations *i.e.* 50, 250 and 500 mM/L of yeast and garlic extracts were used for soaking squash seeds. While seeds of control treatments were soaked in distilled water. In the second season (*i.e.* 1998) at 40 days of plant age samples of the intact plants were carefully collected for the histological

measurements and counts in different plant parts and for the endogenous phytohormones determination in leaves of different treatments.

A- Anatomical studies:

Specimens of roots, stems and leaves were taken from plants aged 40 days from the basal part of the tap root, the 6th basal internode of the main stem and the blade of the 5th leaf. The specimens were then killed and fixed for 48 hours in F. A. A. solution (10 ml formalin: 5 ml glacial acetic acid: 85 ml ethyl alcohol 70%), washed in 50% ethyl alcohol, dehydrated in a series of ethyl alcohols 70, 90, 95 and 100%, infiltrated in xylene, embedded in paraffin wax with a melting point 60-63°C. (Sass, 1967), sectioned at 20 µ, double stained with crystal violet and erythrosin (Jackson, 1976), cleared in xylene and mounted in canada balsam.

The prepared sections were microscopically examined to detect histological manifestation of noticeable responses resulted from treatments with garlic and yeast extracts.

B- Determination of the endogenous phytohormones:

Auxins, gibberellins and cytokinins were biologically determined in leaves as follows:

i- Endogenous auxin - like substances

- Extraction and purification were made according the method of Knight and Bruinsma (1973).
- Chromatogram extract plates were developed according to the method devised by Lobler and Schwarz 1965.
- Bioassay: The wheat (*Triticum*) coleoptile segment straight growth test was used for determination of auxins (Sirois, 1966).

ii- Endogenous gibberellin-like substances:

- Extraction were made according to the method described by Kopecky *et al*., (1975).
- Chromatogram plates were developed by the method described by Sembdner *et al*., (1962).
- Bioassay: The lettuce hypocotyl length bioassay test was used (Frankland and Wareing, 1960).

iii- Endogenous cytokinin-like substances:

Extraction and purification of chromatogram plates were followed up according to the method devised by Rodriques and Houwen (1972).

Bioassay:

The Amaranthus (Pigweed) dark betacyanin promotion bioassay test was used (Conrad and Kohler, 1967).

Standard curves:

Commercial auxin (IAA), gibberellic acid (GA_3) and kinetin were applied in different concentrations in order to obtain standard curves used for comparisons.

Statistical analysis:

- Results of endogenous phytohormones were statistically evaluated according to **Tukey (1953)**.

RESULTS AND DISCUSSION

1- Anatomical studies:

1- Anatomy of the root:

Table (1) shows the effect of the two natural garlic and yeast extracts in concentrations of 50, 250 and 500 ml/L on the histology of squash tap root.

As for garlic extract, the three applied concentrations increased the root diameter by about 65.58, 59.76 and 52.17% more than the control, respectively. This obvious increase in the root diameter was mainly due to the increase in cortex thickness (74.67, 63.33 & 48.00%) and vascular cylinder diameter (63.27, 58.84 & 53.23%) more than those of the control with the three applied concentrations respectively. Also, it could be noticed that the obtained increase was obvious with the low concentration (*i.e* 50 ml/L) more than with the other ones (Table 1 & Figure 1 - b). The increase that existed in cortex thickness was mainly resulted from the slight increase in thickness of exodermis beside the apparent increase in thickness and number of cortical parenchyma layers and mean width of its cells as well. The increase that occurred in the number of cortical parenchyma layers could be an indication for more active longitudinal cell division of the periblem. On the other hand, the increase in vascular cylinder diameter with garlic extract treatments was mainly due to the increase in thickness of cambial region, phloem region, length and width of both secondary xylem bundles and secondary ray, number of vessels/secondary xylem bundle and diameter of secondary xylem vessels. This could indicate more cambial activity due to stimulative effect of garlic extract treatments. However, the number of secondary xylem bundles was not affected. Therefore, the above mentioned results clearly indicate that all applied concentrations of garlic extract enhanced the growth of all tissues comprising the root leading to obvious increase of the root thickness. Also, the effect was inversely proportional to the applied concentration.

With regard to the yeast extract, data in Table (1) reveal that the three applied concentrations caused high increase in the root diameter comparing with the control. Increase values were 30.01, 42.14 and 66.53% for the concentrations of 50, 250 and 500 ml/L, respectively in comparison with the control. The increase in the root diameter could be attributed to the increase in cortex thickness and vascular cylinder diameter. This increase reached 86.00% for

Table (1): Mean counts and measurements (related to the control)of certain histological features in transverse sections through the tap root of squash as affected by garlic and yeast extracts.

Measurements (μ) & counts	Treatments											
		Garlic extract					Yeast extract					
	Control*	50 ml/L		250 ml/L		500 ml/L	50 ml/L		250 ml/L		500 ml/L	
	x	x	%	x	%	x	%	x	%	x	%	x	%
Diameter of whole section	1528.40	2159.6	141.34	2532.2	165.70	2021.4	132.27	1727.1	130.01	1888.2	142.04	2202.2	166.53
Thickness of cortex	110.00	205.80	174.67	220.50	163.23	199.80	143.00	190.00	151.33	208.80	164.67	251.10	186.00
Thickness of epidermis	38.70	46.80	120.93	44.10	113.95	42.30	109.30	42.30	118.47	44.10	113.95	44.10	113.95
Thickness of normal parenchyma layers	96.30	189.00	196.26	176.40	183.18	157.50	163.55	148.05	153.74	168.70	171.83	203.00	214.95
No. of normal parenchyma layers	4.00	5.50	137.50	5.50	137.50	5.00	125.00	5.00	125.00	5.00	125.00	6.50	162.50
x thickness of the cortex parenchyma layers	26.00	34.36	132.15	32.67	125.26	31.50	127.02	29.61	119.48	34.21	137.94	31.85	128.43
Diameter of vascular cylinder	1018.40	1719.0	163.27	1681.2	158.84	1621.8	153.23	1545.5	127.15	1478.6	118.91	1710.0	161.56
x thickness of primary + upper (primary & secondary)	86.40	122.40	141.66	119.70	138.36	106.20	122.92	113.35	128.65	118.80	137.50	109.30	108.35
Thickness of cambial region	29.70	47.70	160.61	45.90	154.55	42.20	145.45	39.35	131.82	42.30	142.42	38.50	196.97
No. of secondary xylem bundles	4.00	6.00	100.00	6.00	100.00	6.00	100.00	6.00	100.00	6.00	100.00	4.00	100.00
x length of secondary xylem bundle	413.10	689.40	166.88	675.00	163.40	661.50	160.13	522.45	126.47	574.20	139.00	577.20	139.25
x width of secondary xylem bundle	315.00	460.80	146.29	446.40	141.71	432.90	137.43	457.20	145.14	472.50	150.00	526.50	167.14
No. of vessels secondary xylem bundle	5.75	8.00	139.13	8.50	147.83	7.25	126.09	6.25	108.70	7.50	130.43	9.50	165.22
Diameter of widest secondary xylem vessel	134.40	124.20	111.87	109.80	105.17	110.40	125.86	127.80	122.41	115.20	118.34	97.20	93.10
x length of secondary medullary ray	405.30	709.50	136.79	759.60	109.77	725.40	154.41	600.75	127.87	643.20	137.36	765.00	159.00
x width of secondary medullary ray	396.00	495.0	125.00	475.20	120.00	484.60	112.37	409.50	105.41	420.60	108.30	462.00	131.87

* Control values are considered as 100%

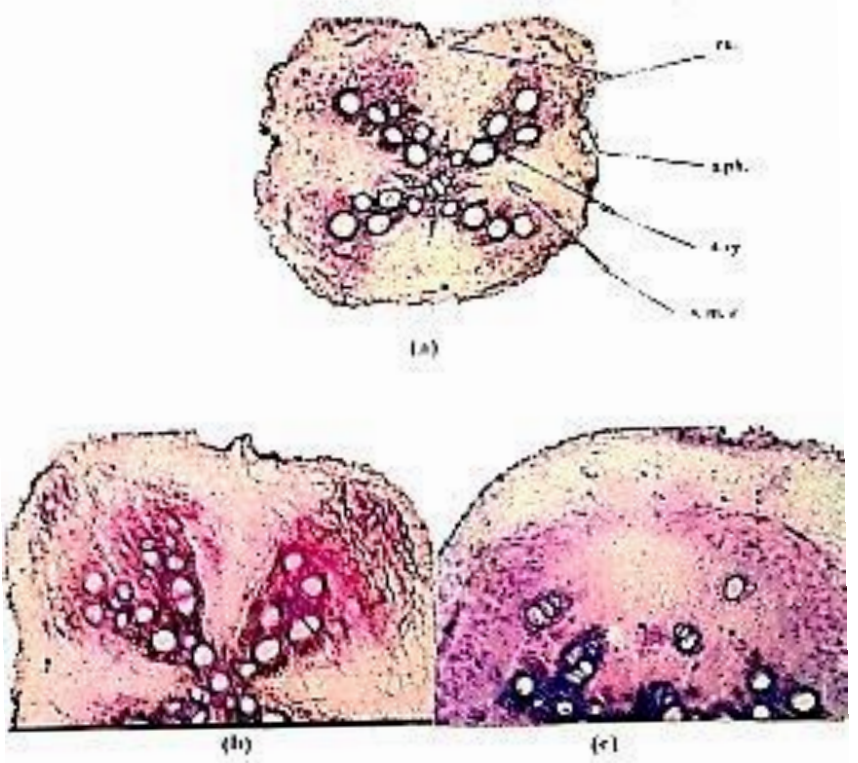


Fig. (1): Transverse sections through the tap root of squash as affected by natural garlic and yeast extracts (50 X).

(a): Of an (control) plot

(b): Of a plant grown from 50 ml% of garlic extract treated seeds

(c): Of a plant grown from 250 ml% of yeast extract treated seeds.

abbr:

ex. = cortex, xph. = secondary phloem region, x. xy. = secondary xylem/vascular region and ray. = secondary ray.

cortex thickness and 61.56% for vascular cylinder diameter with the concentration of 500 ml/L (Table. 1 and Figure 1-c). The increase that existed in cortex thickness was mainly due to the increase in thickness of exodermis thickness and number of the cortical parenchyma layers beside mean width of its cells. While, the increase in vascular cylinder diameter with all applied concentrations of yeast extract was mainly due to the increase in the cambial region activity which led to increases in phloem region thickness, length, width of both secondary xylem bundles and secondary rays, number of vessels/secondary xylem bundle and also diameter of secondary xylem vessels. This increase was directly proportional to the used concentration. The only exception was the reduction that existed in the diameter of secondary xylem vessels with the high concentration (*i.e.* 500 ml/L). However, the number of secondary xylem bundles was not affected. Therefore, different tissues of the root were positively responded due to yeast extract treatments and consequently diameter of the root was increased (*i.e.* root size was increased see El-Desouky *et al*., 1998).

2- Anatomy of the main stem;

Table (2) and Fig. (2) show that, garlic extract treatment with 50, 250 and 500 ml/L led to obvious increase in the stem diameter. The increase was in parallel to the applied concentration. Since, increases were 6.28, 15.81 and 30.22% more than the control with 50, 250 and 500 ml/L respectively. The existed increase in the stem diameter was mainly due to the obvious increase in the stem wall thickness as it reached 51.87% more than the control with the high concentration (500 ml/L). Even though a slight reduction was existed in the hollow pith diameter as it reached 6.69% less than the control at concentration of 500 ml/L. Besides, the increment in the stem wall thickness due to garlic extract treatment was mainly resulted from the increase in many studied histological features such as thickness of epidermis, cortex (cortical collenchyma and parenchyma layers), number of cortical parenchyma layers, thickness and number of parenchymatous pith layers, mean width of cortical and pith parenchyma cells, dimensions (length and width) of vascular bundles, thickness of both outer and inner phloem tissues, thickness of cambial region, thickness of xylem tissue, number of xylem vessels/bundle and also diameter of xylem vessels. These results could indicate the longitudinal cell division was positively affected in meristems giving rise to the ground and vascular tissues. However, the number of cortical collenchyma layers and the number of vascular bundles either the outermost or the innermost were not effected. The only exception was the increase that occurred in the number of innermost vascular bundles (28.57% more than the control) with the high concentration of garlic extract (500 ml/L). Hence, it could be concluded from the above mentioned results that the more thickened stems due to garlic extract treatments resulted from their enhancing effects on nearly all tissues comprising the stem. This enhancing effect was more obvious with the high concentration (*i.e.* 500 ml/L (Table. 2 and Figure. 2 -b).

Table (2): Mean counts and measurements (related to the control) of certain histological features in transverse and longitudinal sections through the middle part of the sixth internode of the main stem of squash as affected by garlic and yeast extracts.

Measurements (μ) & counts	Treatments											
		Garlic extract					Yeast extract					
	Control*	50 ml/L		250 ml/L		500 ml/L		50 ml/L		250 ml/L		500 ml/L	
	x	y	%	y	%	y	%	y	%	y	%	y	%
Diameter of whole section	5202.00	5528.7	106.28	6024.6	115.81	6774.3	130.22	6452.1	124.03	6721.2	129.20	6826.5	131.22
Diameter of hollow pith	1922.40	1885.5	98.08	1811.7	94.24	1793.3	93.31	1878.3	97.71	1706.4	88.76	1570.5	81.69
Thickness of stem wall	1639.80	1821.6	111.09	2106.45	128.46	2490.3	151.87	2286.9	139.46	2507.4	152.91	2826.5	160.26
Thickness of epidermis	21.35	23.40	110.64	25.65	121.28	26.10	123.40	24.75	117.64	25.20	119.15	27.00	127.66
Thickness of cortex	247.50	272.70	103.17	284.40	114.91	302.40	122.18	270.00	109.82	316.80	128.00	360.00	145.45
Thickness of cortical collenchyma layers	170.10	175.50	103.17	177.30	104.23	183.60	107.94	180.00	105.83	191.70	112.70	187.20	110.05
No. of cortical collenchyma layers	8.00	8.00	100.00	8.00	100.00	8.00	100.00	8.00	100.00	8.00	100.00	8.00	100.00
* thickness of cortical collenchyma layers	21.26	21.94	103.17	22.16	104.23	22.95	107.94	22.50	105.83	23.96	112.70	23.40	110.05
Thickness of cortical parenchyma layers	77.40	97.20	125.58	105.30	136.05	138.80	153.49	91.80	118.60	130.50	168.60	172.80	223.26
No. of cortical parenchyma layers	3.00	3.50	116.67	3.50	116.67	4.00	133.33	3.00	100.00	3.50	116.67	4.00	133.33
* thickness of cortical parenchyma layers	25.80	27.77	107.36	30.09	116.63	29.70	115.12	30.60	118.60	37.29	144.53	43.20	167.44
Thickness of pericyclic fibers	22.95	24.30	105.88	27.00	117.65	27.45	119.61	26.55	115.69	27.00	117.65	28.80	125.49
Thickness of parenchymatous pith	541.80	626.40	115.61	738.90	136.38	883.80	163.12	735.80	145.19	880.20	162.46	905.40	167.11
No. of pith parenchyma layers	9.00	10.00	111.11	11.25	125.00	13.00	144.44	11.75	130.50	12.75	141.67	12.50	138.89
# thickness of the pith parenchyma layers	60.20	62.64	104.05	65.68	109.10	67.98	112.92	66.03	109.68	69.04	114.68	72.43	120.32
No. of outermost vascular bundles	5.00	5.00	100.00	5.00	100.00	5.00	100.00	5.00	100.00	5.00	100.00	5.00	100.00
No. of innermost vascular bundles	7.00	7.00	100.00	7.00	100.00	9.00	128.57	7.00	100.00	7.00	100.00	8.50	121.43
Length of outermost vascular bundles	481.50	541.80	112.52	600.30	124.67	648.20	134.71	550.80	114.39	614.70	127.66	658.80	136.82
Width of outermost vascular bundles	576.90	607.50	105.30	676.80	117.32	727.60	126.09	617.40	107.02	578.60	100.47	630.00	109.20
Thickness of outer phloem tissue	88.20	93.60	106.12	98.10	111.22	99.45	112.76	95.40	108.16	102.60	116.33	100.80	114.90
Thickness of inner phloem tissue	118.80	126.00	106.06	134.10	112.88	136.80	115.15	133.20	112.12	145.80	122.73	143.35	120.49
Thickness of fascicular cambium region	39.60	41.85	105.68	45.45	114.73	47.70	120.45	45.00	113.64	46.35	117.05	48.60	122.73
Thickness of xylem tissue	234.90	280.35	119.35	322.65	137.36	362.25	154.21	277.20	118.01	319.95	136.21	366.30	155.94
No. of xylem vessels/bundle	24.50	27.50	112.24	33.00	134.69	40.25	164.29	31.00	126.53	32.50	132.65	34.50	140.82
Diameter of widest vessel	60.75	63.45	104.44	67.50	111.11	66.15	108.89	64.35	105.93	68.40	112.59	69.30	114.07
# length of the cortical parenchyma cell	82.80	99.90	120.65	104.40	126.09	112.5	135.87	78.30	94.56	34.25	89.67	65.70	79.35
* length of pith parenchyma cell	63.90	78.75	123.24	83.45	127.46	89.10	139.44	62.10	97.33	59.40	92.96	55.80	87.32

* Control values are considered as 100%

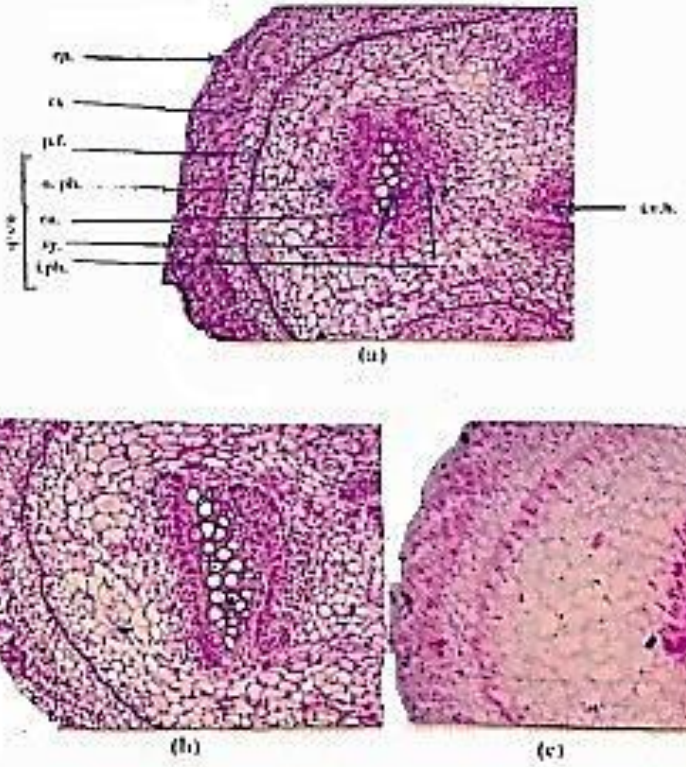


Fig. (3): Transverse sections through the middle part of the six internode of the main stem of squash as affected by natural garlic and yeast extracts (60 X).

(a) Of an untreated plant
(b) Of a plant grown from 500 ml/l of garlic extract treated seeds
(c) Of a plant grown from 500 ml/l of yeast extract treated seeds

able
ep.= epidermis, cx = cortex, p.f. = pericyclic fiber, s.ph = sieve phloem tissue, i.ph. = inner phloem tissue, ca = cambial zone, xy = xylem tissue, v.b = outermost vascular bundle and i.v.b = innermost vascular bundle

On the other hand, Table (2) and Figure, (3-b) clearly show that the three applied concentrations of garlic extract *i.e* 50, 250 and 500 ml/L proportionally increased the mean length of both cortical and pith parenchyma cells. This primitive effect on elongation of parenchyma cells could explain the elongation of internodes due to garlic extract treatments as mentioned before (see El-Desouky *et al*., 1998).

With respect to yeast extract, Table (2) and Figure (2 –c) show that the three applied concentrations increased the internode diameter more than the control by 24.03, 29.20 and 31.23% respectively. This increase in the internode diameter was accompanied with high increase in the stem wall thickness (reached 60.26% more than the control with the concentration of 500 ml/L) and small reduction in the hollow pith diameter (reached 18.31% less than the control with the same concentration). In addition, the increment that appeared in the stem wall thickness was mainly due to the increase in the thickness of epidermis, cortical collenchyma layers, thickness and number of both cortical and pith parenchyma layers, mean width of the cortical and pith parenchyma cells, dimensions of vascular bundles, thickness of outer and inner phloem tissues, cambial region and xylem tissue, number of xylem vessels/bundle and also diameter of vessels as well. This could be an indication for more active longitudinal cell division in meristems giving rise to the ground and vascular tissues. Meanwhile, the number of cortical collenchyma layers and the number of vascular bundles (the outermost or the innermost) were not effected. Exception was that existed with the high concentration of yeast extract (500 ml/L) as it increased the number of innermost vascular bundles by 21.43% more than the control. Thus, it could be concluded that all yeast extract treatments had enhancing effect on nearly all tissues comprising the stem which shared to different extends in increasing the thickness of the stem. This enhancing effect was more obvious with the high concentration treatment *i.e.* 500 ml/L (Table,2 and Fig. 2 -c).

On the other hand, Table (2) and Figure, (3 - c) reveal that the three used concentrations of yeast extract caused but parallel reduction in the mean length of both cortical and pith parenchyma cells. This retarding effect on elongation of parenchyma cells was accompanied with the internode shortness as indicated (see El-Desouky *et al*., 1998).

3- Anatomy of the leaf:

Table (3) show the effect of the natural garlic and yeast extracts with their applied concentrations on the histology of the leaf.

The application of garlic extract with its three assigned concentrations as shown in Table (3) and Figures (4 and 5) led to an increase in the thickness of both midvein and lamina as compared with the control. This increase was proportional to the applied concentration. Since the increase in the midvein was 15.16, 21.47 and 30.07%, meanwhile the increase in the lamina thickness was

Table (3): Mean counts and measurements (related to the control) of certain histological features in transverse sections through the forth leaf on the main stem of squash as affected by garlic and yeast extracts.

Measurements (µ) & count	Treatments											
		Garlic extract					Yeast extract					
	Control*	50 ml/L		250 ml/L		500 ml/L		50 ml/L		250 ml/L		500 ml/L	
			%		%		%		%		%		%
Thickness of central main vein	1056.80	1216.8	115.16	1283.4	121.47	1374.1	130.07	1259.1	119.17	1399.0	132.42	1452.6	137.48
Thickness of uppermost collenchyma tissue	90.00	93.60	104.00	98.10	109.00	99.90	111.00	96.30	107.00	102.60	114.00	97.20	108.00
No. of uppermost collenchyma layers	5.00	5.00	100.00	5.00	100.00	5.00	100.00	5.00	100.00	5.00	100.00	5.00	100.00
Thickness of lowermost collenchyma tissue	82.80	99.00	119.57	95.40	115.22	118.80	143.48	99.00	119.57	103.50	125.00	90.00	108.70
No. of lowermost collenchyma layers	3.00	3.00	100.00	3.00	100.00	3.00	100.00	3.00	100.00	3.00	100.00	3.00	100.00
Thickness of lowermost parenchyma tissue	135.00	163.80	121.33	180.00	133.33	160.20	118.67	171.90	127.33	180.00	133.33	234.00	173.33
No. of uppermost parenchyma layers	3.00	3.00	100.00	3.00	100.00	3.00	100.00	3.00	100.00	3.00	100.00	4.00	133.33
Length of main vascular bundle	4311.0	445.50	103.34	468.90	108.77	502.20	116.49	461.70	107.10	517.50	120.04	538.20	124.84
Width of main vascular bundle	290.80	315.00	112.38	332.10	118.27	318.60	117.46	414.00	147.44	486.00	173.08	640.80	228.2
Thickness of uppermost phloem tissue	42.30	44.10	104.20	44.10	104.26	46.80	110.64	54.90	129.79	67.50	159.57	79.20	187.23
Thickness of lowermost phloem tissue	189.00	196.20	103.81	216.00	104.29	225.00	124.76	184.50	97.62	180.00	95.24	138.60	73.33
Thickness of xylem tissue	199.80	205.20	102.70	208.80	104.50	219.60	109.91	222.30	111.26	270.00	135.14	320.40	160.36
No. of xylem vessels in the main v. bundle	24.50	25.00	102.04	26.00	106.12	28.00	114.29	30.50	124.49	35.00	142.86	42.25	176.57
Diameter of widest xylem vessel	47.70	51.30	107.55	56.70	118.87	46.80	98.11	55.35	116.04	65.70	137.74	76.50	160.38
Thickness of lamina	144.00	175.00	121.53	193.50	134.38	216.00	150.00	184.00	127.78	199.50	138.54	219.25	152.26
Thickness of upper epidermis	12.00	14.25	118.75	15.00	125.00	16.25	135.42	14.00	116.67	15.25	127.08	15.75	131.25
Thickness of lower epidermis	10.25	4.50	112.20	12.00	117.07	13.25	129.27	11.50	112.20	12.25	119.51	12.50	121.95
Thickness of palisade tissue	76.75	90.50	117.92	100.00	130.29	109.75	143.00	93.50	121.82	100.50	130.94	112.50	146.58
No. of palisade layers	2.00	2.00	100.00	2.00	100.00	2.00	100.00	2.00	100.00	2.00	100.00	2.00	100.00
* thickness of the palisade layers	38.38	45.25	117.92	50.00	130.29	54.88	143.00	46.75	121.82	50.25	130.94	56.25	146.58
Thickness of spongy tissue	45.00	58.75	130.55	66.50	147.78	76.75	170.56	65.00	144.44	71.50	158.89	78.50	174.44
No. of spongy layers	3.00	3.00	100.00	3.00	100.00	4.00	133.33	3.00	100.00	3.50	116.67	4.00	133.33
* thickness of the spongy layers	15.00	19.58	130.55	22.17	147.78	19.19	127.93	21.67	144.64	20.43	136.20	19.63	130.87

* Control values are considered as 100%

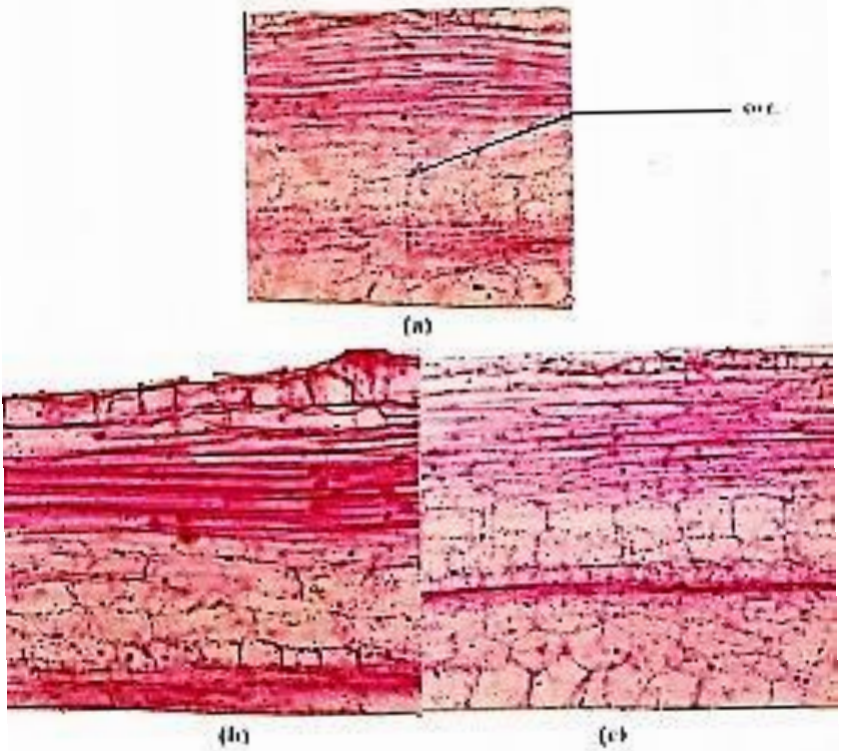


Fig. (3): Longitudinal sections through the middle part of the six internode of the main stem of squash as affected by natural garlic and yeast extracts (125 X).

(a) Of an untreated plant
(b) Of a plant grown from 500 ml/l of garlic extract treated seeds
(c): Of a plant grown from 500 ml/l of yeast extract treated seeds

ab(s)
ex.c. = vertical vascular bundles

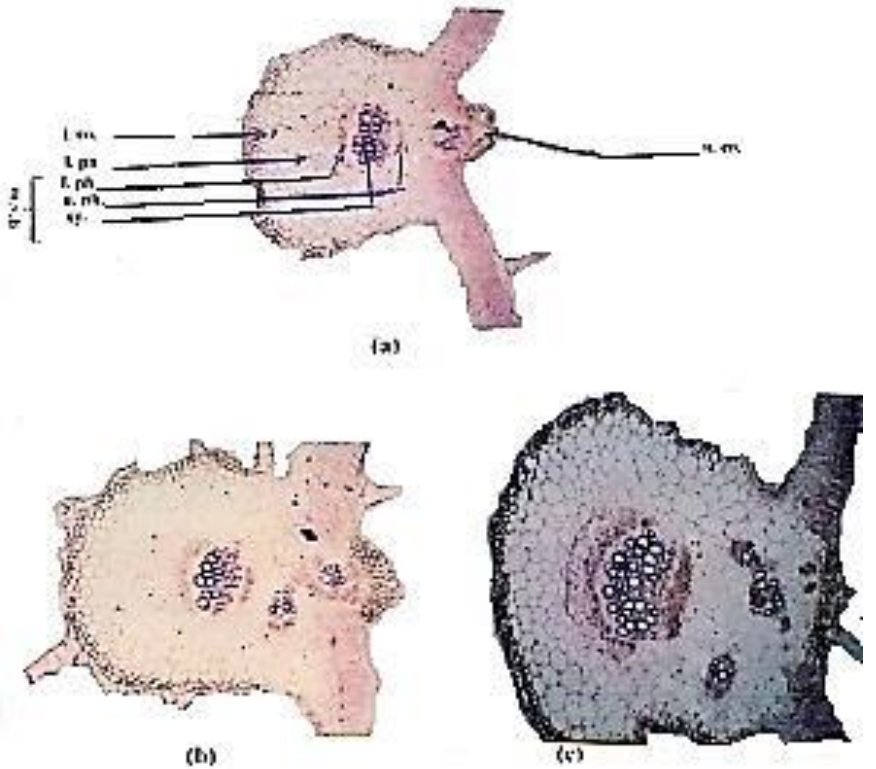


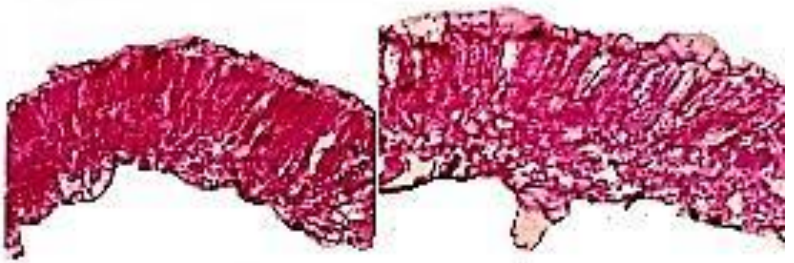
Fig. (4): Transverse sections through the main vein of the fifth leaf of squash as affected by natural garlic and yeast extracts (50 X).

(a): Of an untreated plant
(b): Of a plant grown from 500 ml/l of garlic extract treated seeds
(c): Of a plant grown from 500 ml/l of yeast extract treated seeds

able:

L. co = Lawsonia collenchyma tissue, L. pa = Lawsonia parenchyma tissue, L. ph. = Lawsonia phloem tissue, u. ph = Upper epidermis tissue, xy = xylem tissue, m.v.b. = Main vascular bundle and u. co = Upper collenchyma tissue

(a)



(b) (c)

Fig. (5): Transverse sections through the main vein of the fifth leaf of squash as affected by natural garlic and yeast extracts (150 X).
(a): Of an untreated plant
(b): Of a plant grown from 500 ml/L of garlic extract treated seeds
(c): Of a plant grown from 500 ml/L of yeast extract treated seeds

able
u.e. = upper epidermis, l. e. = lower epidermis, pa. palisade tissue and sp. spongy tissue

21.53, 34.38 and 50.00% more than the control with the concentrations of 50, 250 and 500 ml/L, respectively. For the noticed increment in the midvein thickness it could be attributed to the increase in many histological features such as thickness of both uppermost and lowermost collenchyma tissues, thickness of lowermost parenchyma tissue, dimensions of main vascular bundle, thickness of both uppermost and lowermost phloem tissues, xylem tissue thickness and also number and diameter of xylem vessels in the main vascular bundle. This increase was more obvious with the high concentration *i.e.* 500 ml/l, Table (3) and Figure (4 b). The only exception was the slight reduction that existed in the diameter of xylem vessels (1.89% less than the control) at the concentration of 500 ml/L. While, the number of both uppermost and lowermost collenchyma layers and the number of lowermost parenchyma were not affected. But for the increment that occurred in the lamina thickness due to garlic extract treatments was accompanied with the increase in the thickness of its tissue components *i.e.* mean width of both palisade and spongy cells (Table,) and Figure 5 - b). While, thickness of : both upper and lower epidermis, palisade and spongy tissues, also the number of both palisade and spongy layers were not affected. The only exception was that increase existed in the number of spongy layers with the high concentration (500 ml/L). Since, the number of spongy layers was increased by 33.33% more than the control. These results could indicate that all tissues comprising the leaf was positively affected due to garlic extract treatments. This positive effect was more obvious with the high concentration *i.e.* 500 ml/L (Table, 3 and Figures. 4 - b and 5 - b).

The situation with the yeast extract treatments was greatly similar to that of garlic extract treatments. Since, as shown in Table (3) and Figures (4 - c and 5 - c) the three used concentrations of yeast extract *i.e.* 50, 250 and 500 ml/L caused but apparent increment in the thickness of both midvein and lamina comparing with the control. This increment was parallel to the applied concentration, as the increament was 19.17, 28.62 and 37.48% for the thickness of midvein and 27.78, 38.54 and 52.26% for the thickness of lamina with the concentration of 50, 250 and 500 ml/L, respectively. As for the increase that existed in the midvein was mainly due to the slight increase in the thickness of both uppermost and lowermost collenchyma tissues, beside the high increase in thickness of lowermost collenchyma tissues, length and width of main vascular bundle, thickness of uppermost phloem tissue and xylem tissue and also number and diameter of xylem vessels in the main vascular bundle. This increase was more obvious with the high concentration *i.e.* 500 ml/L (Table, 3 and Figure, 4 - c). Even though reduction occurred in the thickness of lowermost phloem tissue, Meanwhile, the number of both uppermost and lowermost collenchyma layers and the number of lowermost collenchyma layers were not affected. The only exception was the increase that appeared in the number of lowermost collenchyma layers (was 33.33% more than the control) with the concentration of 500 ml/L. With regard to the apparent increase in the lamina thickness due to yeast extract treatments was mainly resulted from the increase in thickness of both upper and lower epidermis, thickness of palisade tissue (increased with

46.58% at concentration of 500 ml/L), thickness and number of spongy tissue layers (they increased 74.44 and 33.33% with the concentration of 500 ml/L) and also mean width of both palisade and spongy cells, (Table. 3 and Figure, 5 - c). Hence, these results could reveal that the more thickened leaf due to yeast extract treatments was results from their enhancing effect on all tissues comprising the leaf. This enhancing effect was more obvious with the high concentration i.e. 500 ml/L (Table. 3 and Figures, 4 - c and 5 - c).

Generally, it could be concluded that the two natural extracts i.e. garlic and yeast extracts with their applied concentrations had to a great extend similar enhancing effect on nearly all tissues compising the root, stem and leaf of squash. This enhancing effect was mostly more obvious with yeast extract treatments than with garlic extract treatment and also it was directly proportional to the applied concentration except in root since it was inversely proportional to the applied concentration of garlic extract only.

II- Endogenous phytohormones:

a- Auxins:

As shown in Fig. (7), soaking squash seed before sowing in different applied concentrations i.e. 50, 250 and 500 ml/L of either garlic or yeast as natural extracts significantly increased auxins activity in leaves of treated plants. Also, it could be noticed that, the concentrations of 50 and 250 ml/L exhibited the highest activities of auxin like-substances that was true in case of both garlic and yeast extracts. Moreover, the auxins activity was higher in garlic treatments than in case of the yeast treatments.

b- Gibberellins:

Fig. (8) clearly indicates that the activity of gibberellin like-substances positively responded to garlic and yeast extract treatments. Since, the activity was very low in case of the control but significantly increased with different concentrations of both garlic or yeast treatments. Also, garlic extract showed higher gibberellins activity more than those of the yeast.

c- Cytokinins:

As indicated in Fig. (9) it is evident that cytokinins positively responded to the different extract treatments. Also, both garlic and yeast treatments being have the same ability for increasing cytokinins creation in leaves of treated plants.

Generally, increment of endogenous hormones in squash plants that existed in the present study could be interpret both the obtained modifications in different studied histological features (Tables, 1, 2 & 3 and Figs. 1 to 5) and the improvement of growth and yield that was found before (see El-Desouky *et al*., 1998).

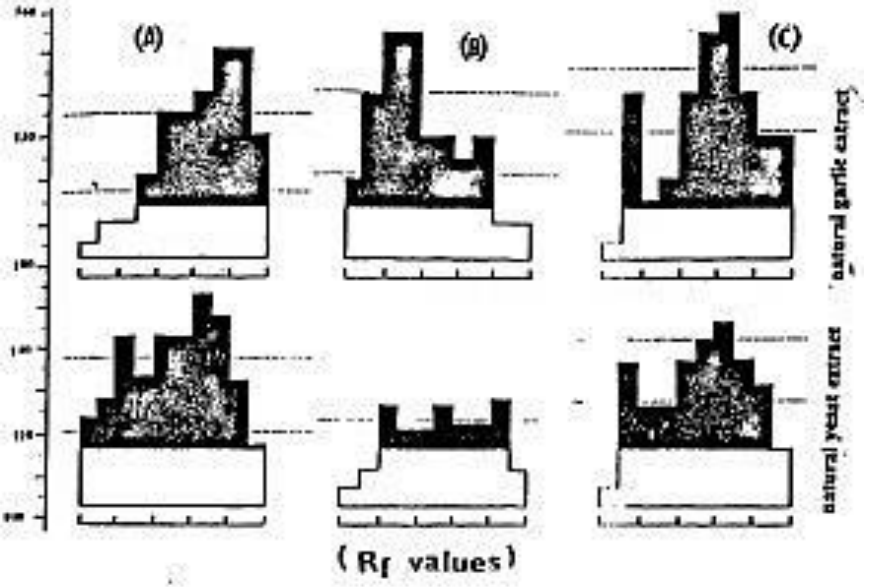


Fig (6) : Endogenous phytohormones activities : i.e. auxins , gibberellins and cytokinins in natural garlic and yeast extracts .
A- auxins B-Gibberellins C- cytokinins
The least significant differences between any two readings is 1-4 mm for auxins ; 1.6 for gibberellins and 1.3 for cytokinins (the base of the black parts) .

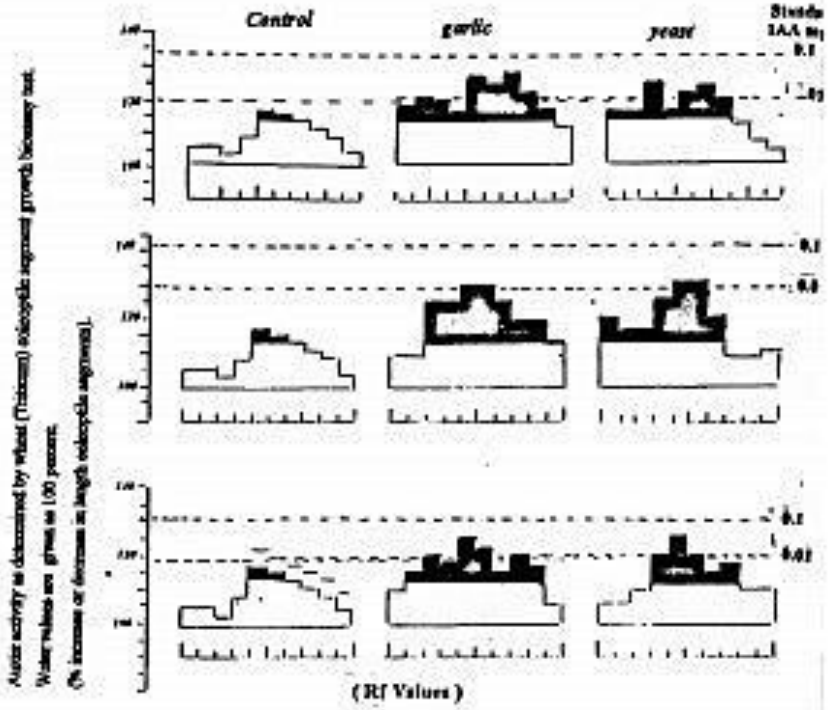


Fig .(7): Effects of the natural extracts of garlic and yeast on the endogenous levels of auxin-like substances.

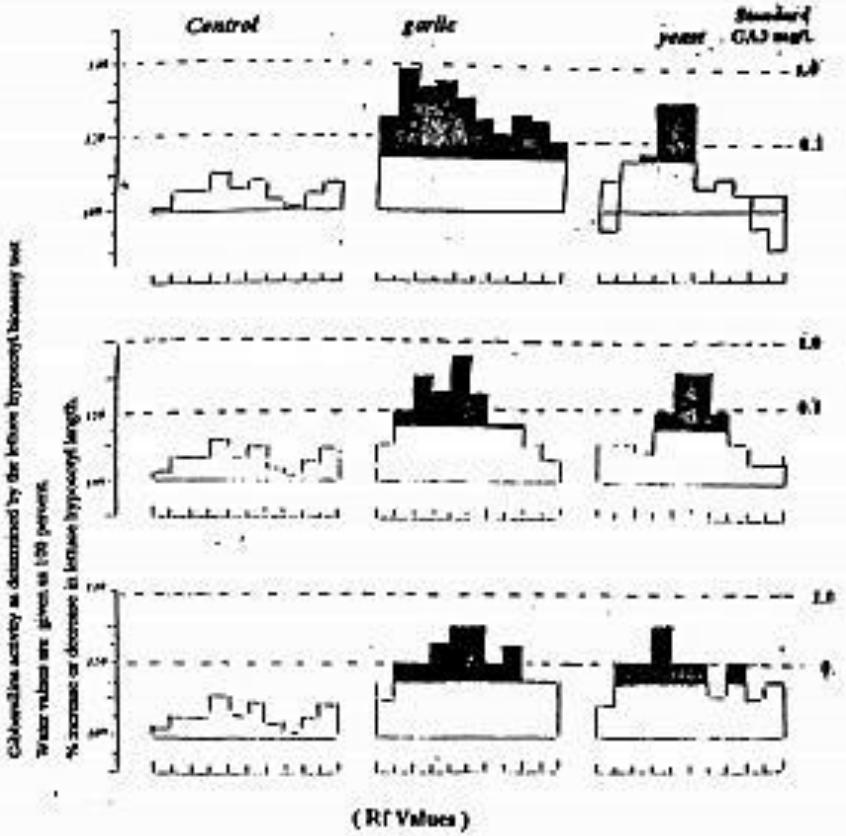


Fig.4b: Effects of the natural extracts of garlic and yeast on the endogenous levels of Gibberellins- like substances in leaves of squash plants.

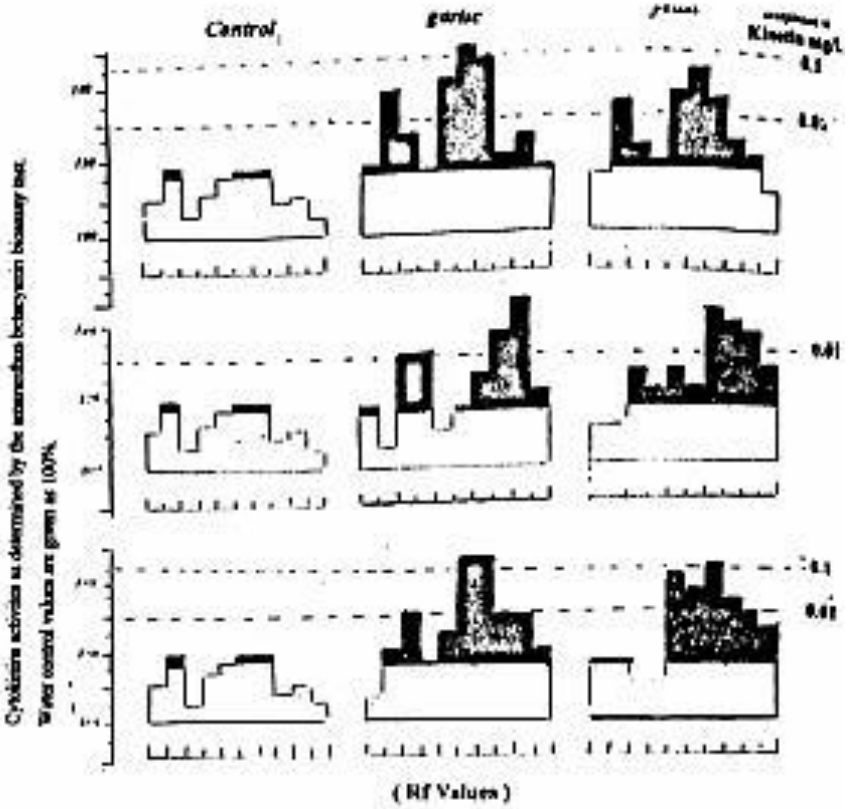


Fig .(9): Effects of the natural extracts of garlic and yeast on the endogenous levels of cytokinins like-substances in leaves of squash plants.

Therefore, it is reasonable to propose that these aspect are-like most other hormonally controlled processes-not dependent on one single hormone but rather on a delicate and quantitative interactions between cytokinins and other phytohormones, particularly the auxins. Of these are the interaction of auxin and cytokinin in cell division and root formation, since the discovery of cytokinins by Miller *et al*., (1955), Devlin and Withem (1983), Ridge, (1993).

Here, of interest then, the present study reports a pioneer work about the increase in the thickness of cambial region in the roots of squash plants grown from pre-soaked seeds in natural garlic or yeast extracts (Table (1) and Fig. 1). The increases in the thickness of cambial region were 60.61, 54.55 & 45.45% for garlic extract treatments and 31.82, 42.42 & 96.97% more than the control for yeast extract treatments with 50, 250 & 500 ml/L, respectively. Hence, all other histological aspects in roots e.g. diameter of vascular cylinder, diameter of the whole section, length and width of secondary mysicle were also positively affected.

Therefore, vigorus growth of the root system and improvement of vegetative growth and the yield and fruit characters were obtained (see El-Desouky *et al*., 1998).

In this respect, there is good evidence that gibberellins and auxins interact to stimulate cambial growth (Pharis, 1976); and there is evidence that these hormones or their active forms may originate in the root. These common growth forms in close association with roots are good evidence that a root-derived factor interacts with the products synthesized in the shoots to regulate cambial growth (Pharis and Reid 1985).

Also, auxins have been positively identified in the cambial region and many strong correlation between auxin and cambial activity have been reported (Little and Wareing 1981 and Savidge 1983).

In addition, the cambial meristem that produce phloem and xylem cells appeared to be controlled and directly regulated by endogenously produced hormones (Pharis and Reid 1985).

Furthermore, cytokinins has been admitted to be produced in root meristems and to some extent has a regularly role in root growth (Ridge *et al*., 1993). That is why, root system of squash exhibited high significant increase regarding its size (see El-Desouky *et al*., 1998) and many alterations were existed regarding its histological feature (present part of the study).

Besides, increment of endogenous hormones creature in squash plants by both natural extract treatments were accompanied with female flowers increases and improving their sink ability as well (i.e. led to yield increases).

Hence, the usage of such natural plant extract having the ability for increasing endogenous hormones creating might provide a direct means not only for avoiding or reducing the excessive use of both chemical fertilizers and synthetic plant growth regulators but also to supply basic information regarding how plants grow and develop and to undertake research programs designed specifically to increase the productivity of plants.

REFERENCES

Conrad, K. and Kohler, K.H. (1967): On the specificity of a phytokinin test due to increased formation of betacyanin in amaranthus seedling. In Wachstumsregulatoren bei Pflanzen E. Libbert and D. Steyer, Eds. Rostock, 657.

Devlin, M. and Witham, H. (1983): Plant Physiology 4th Ed. Publishers Willard Grant Press Boston.

El-Desouky, S.A.; Wanas, A.L.A. and Khedr, Z.M.A. (1998): Utilization of some natural plant extracts (of garlic and yeast) as seed – soaked materials to squash (*Cucurbita pepo* L.). I- Effect on growth, sex expression and fruit yield & quality. Annals Of Agric. Sc., Moshtohor, Vol. 36(2): 839-854.

Frankland and Wareing, P.F. (1960): Effect of gibberellic acid on Hypocotyl growth of lettuce seedlings. Nature. 185 – 255.

Jackson, G. (1976): Crystal violet and erythrosin in plant anatomy. Stain Tech., 1: 33 – 34.

Knight, E. and Brainsma, J. (1973): A rapid, sensitive and accurate determination of indolyl-3-acetic acid. Phytochemistry. 12: 753-755.

Kopecky, F.; Sebanek, J. and Blazkova, J. (1975): Time course of the changes in the level of endogenous growth regulators during the stratification of the seeds of the "Panenska Cheske". Biol., Plant, 17: 31-37.

Lahler, H and Schwarz, V. (1963): Thin layer chromatography. CSAV, Praha.

Little, C.H.A. and Wareing, P.F. (1981): Control of cambial activity and dormancy in *Picea sitchensis* by indole – 3-yl- acetic and abscisic acids. Can. J. Bot., 59: 1480 – 1493.

Miller, C.O.; Skoog, F.; Saltza, M.H. and Strong, F.M. (1955): Kinetin a cell division factor from deoxyribonucleic acid. J. Am. Chem. Soc., 77, 1329.

Nickell, H.G. (1981): Plant growth regulators, Agricultural uses. , Springier – Verlage. New York, Heidelberg, Berlin.

Noggle, G.R. and Fritz, G.J. (1992): Introductory plant physiology. 2nd Ed. Prentice Hall of India. New Delhi – 110001.

Pharis, R.P. (1976): Manipulation of flowering in conifers through the use of plant hormones. In. Miksche, J. P. (ed.) Modern methods in forest genetic. Springier Berlin, Heidelberg New York, pp. 265- 282.

Pharis, R.P. and Reid, D.M. (1985): Hormonal Regulation of Development III. Role of Environmental Factors. Springier – Verlage. Berlin Heidelberg New York Tokyo.

Ridge, I.; Murphy, P.; Bell, M. and Parker, P. (1993): Plant Physiology. Biology form and Function. Edit., Irene Ridge. Hodder and Stoughton. E.T.D. The open University, UK.

Rodriques, P.A.S. and Hourcn, P.S.W. (1972): cytokinins and bacterial symbiosis at *Alnus* species. Pflanzenphysiol. 68: 170-177.

Sass, J.E. (1967): Botanical Microtechnique. Oxford and IBH Publishing Co., Calcutta, 3rd Ed., pp. 228.

Savidge, R.A. (1983): The role of plant in higher plant cellular differentiation. II- Experiments with the vascular cambium and sclereid and tracheid differentiation in the pine, Pinus contorta. Histochem J. 15: 447 – 466.

Sembdner, G.; Gross, R. and Schreiber, K. (1962): Die Dunnschicht chromatographie von Gibberellin. Experientia. 18: 584 – 585.

Sinnis, J.C. (1966): Studies on growth regulators. I- Improved avena coleoptile elongation for auxin. Plant Physiol., 41: 1308.

Tuky, J.W. (1953): Some selected quick and easy methods of statistical analysis. Trans. N.Y., Acad. Sci., 16: 88 – 97.

استخدام بعض المستخلصات النباتية الطبيعية (مستخلصات من الثوم والخميرة) في تلقيح بذور الكوسة
٢- التأثير على التركيب الداخلي والمحتوى الهرموني

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أجريت هذه التجارب في أعشاش في موسمي ١٩٩٦ ، ١٩٩٧ وقد تم تلقيح بذور الكوسة صنف "اسكندراني" في المستخلصات الطبيعية لكل من الثوم و الخميرة وذلك بالتركيزات ٥٠ ، ٢٥٠ ، ٥٠٠ مل/لتر من كل منها وذلك لمدة أربعة ساعات. وفي الموسم الثاني (١٩٩٧) وبعد أربعون يوما من الزراعة أخذت العينات من الجذور والسيقان والأوراق للدراسة التشريحية وكذلك من الأوراق لدراسة المحتوى الهرموني الداخلي وقد كانت أهم النتائج المتحصل عليها كالآتي:

أولاً التركيب الداخلي:

أدت المعاملة بكل من المستخلص الطبيعي للثوم والخميرة في جميع التركيزات المستخدمة إلى زيادة كبيرة في معظم الصفات التشريحية التي تم دراستها في كل من الجذور والسيقان والأوراق.

- حيث أدي كلا المستخلصين إلي زيادة كبيرة في قطر القطاع في كل من الجذور والساق وزيادة واضحة في سمك طبقة الكامبيوم في الجذور والسيقان وقد أدت زيادة سمك طبقة الكامبيوم إلي زيادة كبيرة في الصفات التشريحية الأخرى للجذور والسيقان.

- وقد ادت كذلك المعاملات المختلفة إلي زيادة واضحة في سمك كل من النسيج الإسفنجي والكلورنشيمي في أوراق النباتات المعاملة.
- وقد وجد أن كل من عدد الحزم الوعائية في الجذر والساق لم يتغير في جميع المعاملات وكذلك عدد طبقات النسيج الإسفنجي والكلورنشيمي في أوراق النباتات المعاملة.

ثانيا المحتوي الهرموني الداخلي:

أدي كل من مستخلص الثوم والخميرة إلي زيادة كبيرة في النشاط الهرموني الداخلي للسيتوكينينات والجبريللينات والأكسينات وعدم ظهور أي نشاط للمثبطات الطبيعية للمجاميع الهرمونية الثلاثة التي تم تقديرها.

وقد اتضح من هذه الدراسة بجزئيها الأول والثاني إلي إمكانية استخدام مثل هذه المستخلصات الطبيعية لمعاملة بذور الخضروات التي تأكل طازجة وذلك لتحسين نموها وزيادة المحصول من الثمار وتحسين خواصها وكذلك تقليل استخدام الأسمدة الكيماوية - حيث أدت المعاملات بكل من المستخلصين إلي زيادة حجم المجموع الجذري وزيادة القدرة الامتصاصية للنباتات المعاملة وبالتالي زيادة القدرة التخزينيه مما انعكس علي زيادة محصول الثمار مع تحسين خواصها .