RESULTS

EXPERIMENTAL RESULTS

Part I: Pathological Studies:

I.1- Isolation and identification of the causal organism(s):

Samples of diseased watermelon (*Citrullus lanatus*) plants showed wilt symptoms **(Photo, 1)** were collected from different localities, namely Baltem and Kafr El-Sheikh (Kafr El-Sheikh governorate), Sadat and Khatatba (Minufiya governorate), Abo-Soyer and Salhia (Ismailia governorate), Kom-Hamada and Badr (El-Bihira governorate) and Kafr El-Batekh (Domyat governorate), for isolation of wilt pathogen(s). and were identified at the Agric. Botany Dept., Fac. Agric., Moshtohor. The identification of the isolated fungi was kindly confirmed by Mycology and Plant Disease Survey Department, Plant Pathology Res. Inst., Agric. Res. Cent., Giza, Egypt. The obtained results are shown in **Table (1).**

Data, **Table (1)** show that the fungi *Fusarium oxysporum* (19 isolates), *Rhizoctonia solani* (6 isolates), *Fusarium solani* (4 isolates), *Macrophomina phaseolina* (5 isolates) and *Verticillium dahlia* (7 isolates) were isolated from the wilted plants collected from the localities mentioned above.

It is clearly shown that *F. oxysporum* was the most frequently isolated fungus (9 localities) followed by *V. dahlia* (6 localities), *M. phaseolina* (4 localities), *R. solani* (3 localities) and *F. solani* (3 localities). From all the isolated fungi, *F. oxysporum* was chosen to study its pathogenic capabilities.

Table (1): Occurrence and frequency of fungi isolated from diseased plants collected from different localities of ARE.

Isolated fungi	Localities								
	Kafr El-Sheikh		Minufyia		Ismailia		El-Bihira		Domyat
	Baltem	Kaf El-Sheikh	Sadat	Khatatba	Abo-Soyer	Salhia	Kom-Hamada	Bader	Kafr El-Batekh
F. oxysporum	2	3	4	1	2	1	2	1	3
R. solani	1	--	--	2	--	1	--	--	2
F. solani	--	--	1	--	1	2	--	--	--
M. phaseolina	--	--	--	2	1	1	1	--	--
V. dahlia	1	2	--	1	1	--	1	1	--

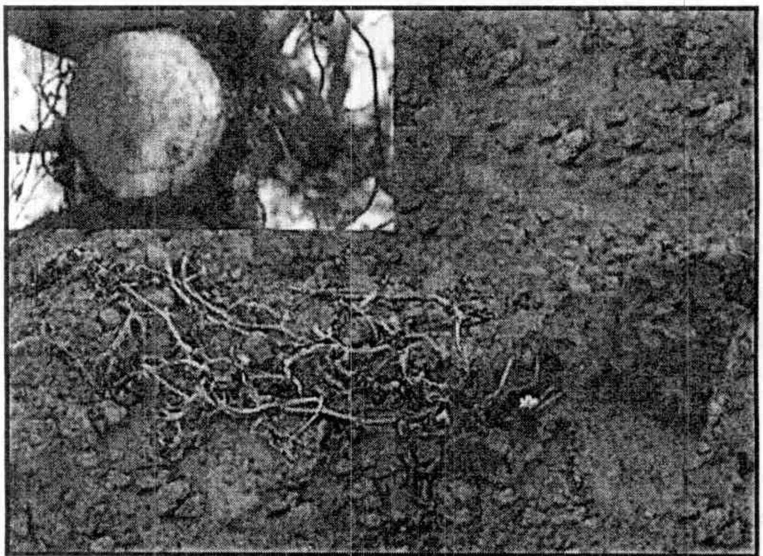


Photo (1): Watermelon plants (Giza 1 cv.) from Baltem (Kafr El-Sheikh governorate) showing severe symptoms of Fusarium wilt.

I.2- Pathogenicity test:

Data in **Table (2)** indicate that all of the tested isolates of *F. oxysporum* were pathogenic to watermelon. They caused significant increase in incidence of pre-emergence damping off (20.0-82.5%) and post- emergence damping off (7.5-25.5%) and significant decrease in healthy survivals (5.0-57.5%) compared with control that showed 5.0, 0.0 and 95.0% for the three parameters, respectively.

As for pre-emergence damping off, isolate No. 7 from Minufyia governorate (Sadat) (82.5%) and isolate No. 2 from Kafr El-Sheikh governorate (Baltem) (75.0%) were the most aggressive without significant difference in between followed by isolates No. 18 (Domyat, Kafr El-Batekh) (62.5%) and No.15 (El-Bihira, Kom-Hamada) (60.0%). While, isolates No. 10 (Minufyia, Khatatba) and No. 14 (El-Bihira, Kom-Hamada) showed the lowest significant percentages for pre-emergence damping-off *i.e.* 20 and 25%, respectively compared with 5.0% in control.

Regarding with post-emergence damping-off, isolates No.13 from Ismailia (Salhia), No.6 from Minufyia (Sadat), No.17 & No.19 from Domyat (Kafr El-Batekh), No.11 from Ismailia (Abo-Soyer), No.1 from Kafr El-Sheikh (Baltem and No.3 from Kafr El-Sheikh (Kaf El-Sheikh) were the most severe isolates (20.0-25.5%) without significant differences in between. While, the lowest significant percentage was noticed with isolate No. 10 (7.5%) compared with 0.0% in control.

Table (2): Effect of different isolates of *Fusarium oxysporum* obtained from different localities on percentage of damping-off on Sugar Baby watermelon cultivar.

No.	Isolates District (Locality)	Damping-off % Pre	Damping-off % Post	Total death	Healthy seedlings
1	Kafr El-Sheikh (Baltem)	47.5	22.5	70.0	30.0
2	Kafr El-Sheikh (Baltem)	75.0	17.5	92.5	7.5
3	Kafr El-Sheikh (Kaf El-Sheikh)	40.0	20.0	60.0	40.0
4	Kafr El-Sheikh (Kaf El-Sheikh)	47.5	17.5	65.0	35.0
5	Kafr El-Sheikh (Kaf El-Sheikh)	40.0	15.0	55.0	45.0
6	Minufyia (Sadat)	35.0	25.0	60.0	40.0
7	Minufyia (Sadat)	82.5	12.5	95.0	5.0
8	Minufyia (Sadat)	50.0	15.0	65.0	35.0
9	Minufyia (Sadat)	47.5	15.0	62.0	37.5
10	Minufyia (Khatatba)	20.0	7.5	27.5	72.5
11	Ismailia (Abo-Soyer)	45.5	22.5	70.0	30.0
12	Ismailia (Abo-Soyer)	55.0	12.5	67.5	32.5
13	Ismailia (Salhia)	50.0	25.5	75.0	25.0
14	El-Bihira (Kom-Hamada)	25.0	17.5	42.5	57.5
15	El-Bihira (Kom-Hamada)	60.0	17.5	77.5	22.5
16	El-Bihira (Bader)	37.5	12.5	50.0	50.0
17	Domyat (Kafr El-Batekh)	42.5	22.5	65.0	55.0
18	Domyat (Kafr El-Batekh)	62.5	12.5	75.0	25.0
19	Domyat (Kafr El-Batekh)	52.5	22.5	75.0	25.0
	Control	5.0	0.0	5.0	95.0
	L.S.D. at 0.05	8.04	7.14	7.78	7.77

Concerning healthy survivals, isolates No. 7 and No.2 produced the highest significant decrease in percentages of the healthy survivals (5.0-7.5%) followed by isolates No.15 (El-Bihira, Kom-Hamada) (22.5%), No.13 (Ismailia, Salhia), No.18 (Domyat, Kafr El-Batekh) and No.19

(Domyat, Kafr El-Batekh), being 25.0%. While, isolate No.10 produced the lowest significant decrease (72.5%) compared with control (95.0%).

Data proved also that the isolates from the same district were significantly varied in their pathogenic abilities. For example, the ability of the five isolates (Nos. 1 - 5) obtained from Kafr El-Sheikh district in causing pre-emergence was varied (40.0 - 75.0%), post-emergence (15.0 -22.5%) and healthy survivals (7.5 - 45.0%). Similarly, the five isolates obtained from Minufyia governorate *i.e.* No.6-10 caused 20.0-82.5% pre-emergence, 7.5-25.0% post-emergence and produced 5.0-72.5% healthy survivals.

In general, the obtained results showed that all isolates of *F. oxysporum* were pathogenic and significant variations were detected in their pathogenic abilities even between those isolated from diseased plant materials collected from the same governorates. The isolates No. 7 from Minufyia (Sadat) and isolate No. 2 from Kafr El-Sheikh (Baltem) were the most severe in this respect.

I.3- Inoculum potential:

Effect of inoculum potential of the most severe isolates of *F. oxysporum i.e.* No. 2 and No.7 was tested under greenhouse conditions. The inocula were used at rates of $1x10^3$, $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$ and $1x10^8$ conidia/ml. Disease assessment was carried out as mentioned under materials and methods. The obtained results shown in **Table (3)** and **Fig. (1)** confirm the significant pathogenic effect of the two isolates of *F. oxysporum*. It is clearly indicate that increasing inoculum levels of both isolates from $1x10^3$ up to $1x10^8$ conidia/ml significantly increased % pre- & post-emergence damping off and decreased % survival. At the lowest inoculum level ($1x10^3$/conidia/ml), isolate No. 2 recorded 15.0, 10.0 & 75% while, isolate No.7 recorded 17.5, 10.0 and 72.5% for pre-, post-emergence and healthy survivals, respectively. However, at the highest inoculum level ($1x10^8$/conidia/ml) isolate No. 2 recorded 70.0, 25.0 and 5.0% while, isolate No.7 recorded 75.0, 17.5 and 7.5% for pre-, post-emergence and healthy survivals, respectively.

Table (3): Effect of different inoculum levels of the two isolates (No. 2 and 7) of *Fusarium oxysporum* on percentages of pre- and post-emergence damping-off and healthy survivals of watermelon Giza 1 cultivar.

Inoculum rates	% Disease incidence					
	Isolate No.2			Isolate No.7		
	Pre	Post	Healthy	Pre	Post	Healthy
$1x10^3$	15.0e	10.0b	75.0	17.5e	10.0ab	72.5
$1x10^4$	17.5e	12.5b	70.0	20.0e	15.0a	65.0
$1x10^5$	30.0d	07.5bc	62.5	30.0d	12.5a	57.5
$1x10^6$	37.5c	10.0b	52.5	50.0c	07.5b	42.5
$1x10^7$	57.5b	15.0b	27.5	62.5b	15.0a	22.5
$1x10^8$	70.0a	25.0a	05.0	75.0a	17.5a	07.5
Control	00.0f	00.0c	100.0	00.0f	00.0b	100.0
L.S.D.	4.50	7.55	5.13	4.68	5.69	7.60

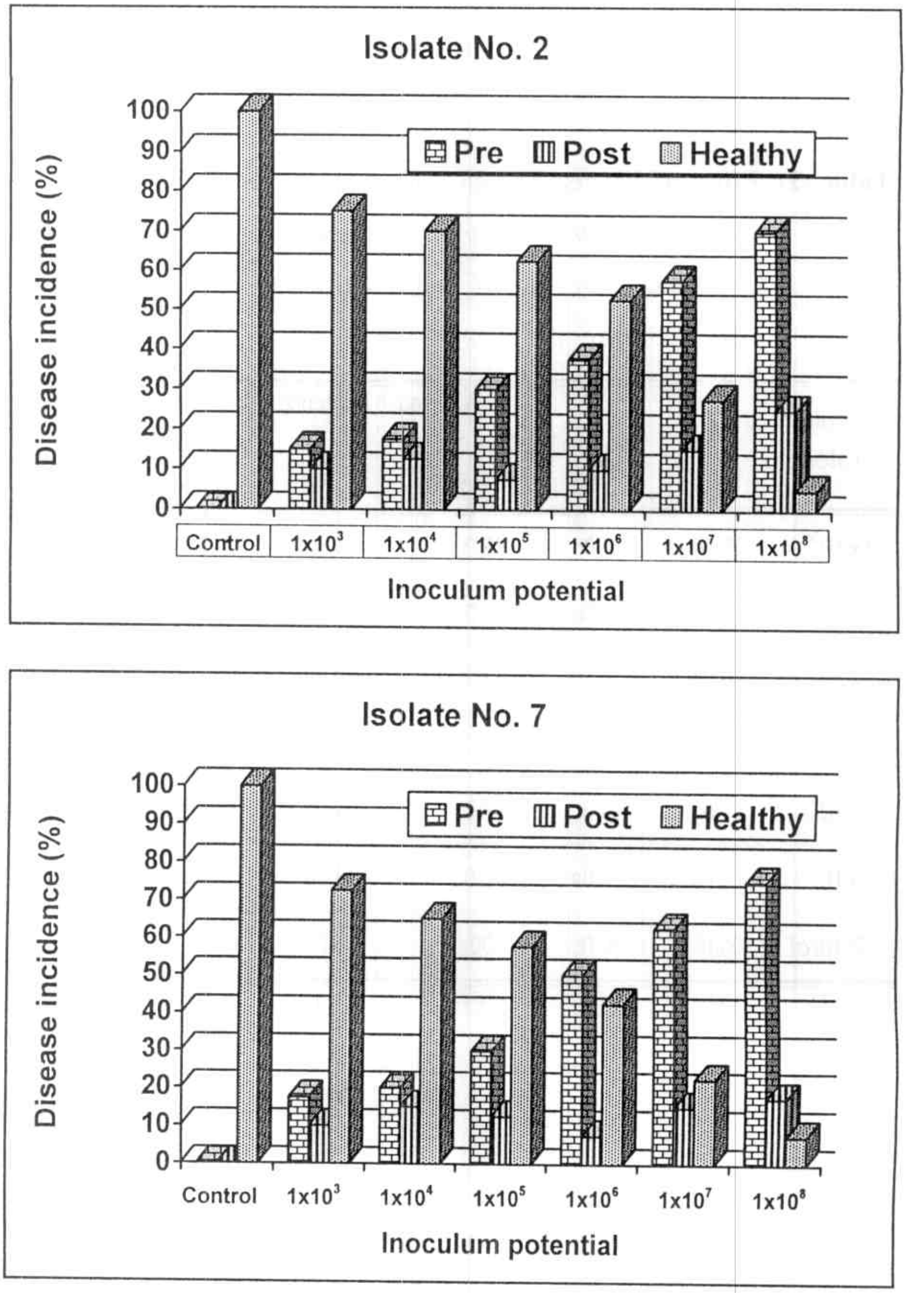


Fig (1): Effect of different inoculum levels of the two isolates (No. 2 and 7) of *Fusarium oxysporum* on percentages of pre- and post-emergence damping-off and healthy survivals of watermelon Giza 1 cultivar.

I.4- Host range:

Data in **Table (4)** and **Fig. (2)** reveal that among the tested host plants, watermelon cultivars only were susceptible to infection with the two *Fusarium oxysporum* isolates (No. 2 & No.7). The watermelon cultivar Sugar Baby was more susceptible to both isolates than Giza 1 cv. In the cultivar Sugar Baby, isolate No.2 recorded 67.5, 25 and 7.5% whereas, isolate No.7 recorded 27.5, 10.0 & 62.5% for pre-emergence, post-emergence and healthy survivals, respectively. The comparative values for pre-emergence, post-emergence and healthy survivals in Giza 1 cv. were 37.5, 17.5 and 45.0% (isolate N.2) and 62.5, 30.0 & 7.5% (isolate No.7), respectively. The tested isolates of *Fusarium oxysporum* were not able to infect cucumber (Beta Alfa cv.) and muskmelon, (Honeydew cv.) and cause appreciable percentage in pre-emergence stage, only (5.0%) of Squash (Eskandrany cv.). These results emphasized specificity of the tested isolates of *Fusarium oxysporum* isolates on watermelons only. Consequently, it was identified as *F. oxysporum* f.sp. *niveum*.

Table (4): Effect of *Fusarium oxysporum* isolates No. 2 & 7 on damping-off disease incidence on different cucurbitacious plants.

	Isolate No.2			Isolate No.7		
Hosts	**Pre**	**Post**	**Healthy**	**Pre**	**Post**	**Healthy**
Watermelon Giza 1	37.5	17.5	45.0	27.5	10.0	62.5
Watermelon Sugar Baby	67.5	25.0	7.5	62.5	30.0	7.5
Squash Eskandarny	5.0	0.0	95.0	0.0	0.0	100.0
Cucumber Beta Alfa	0.0	0.0	100.0	0.0	0.0	100.0
Muskmelon Honeydew	0.0	0.0	100.0	0.0	0.0	100.0
L.S.D. at 5%	11.34	4.87	10.71	10.40	8.44	10.14

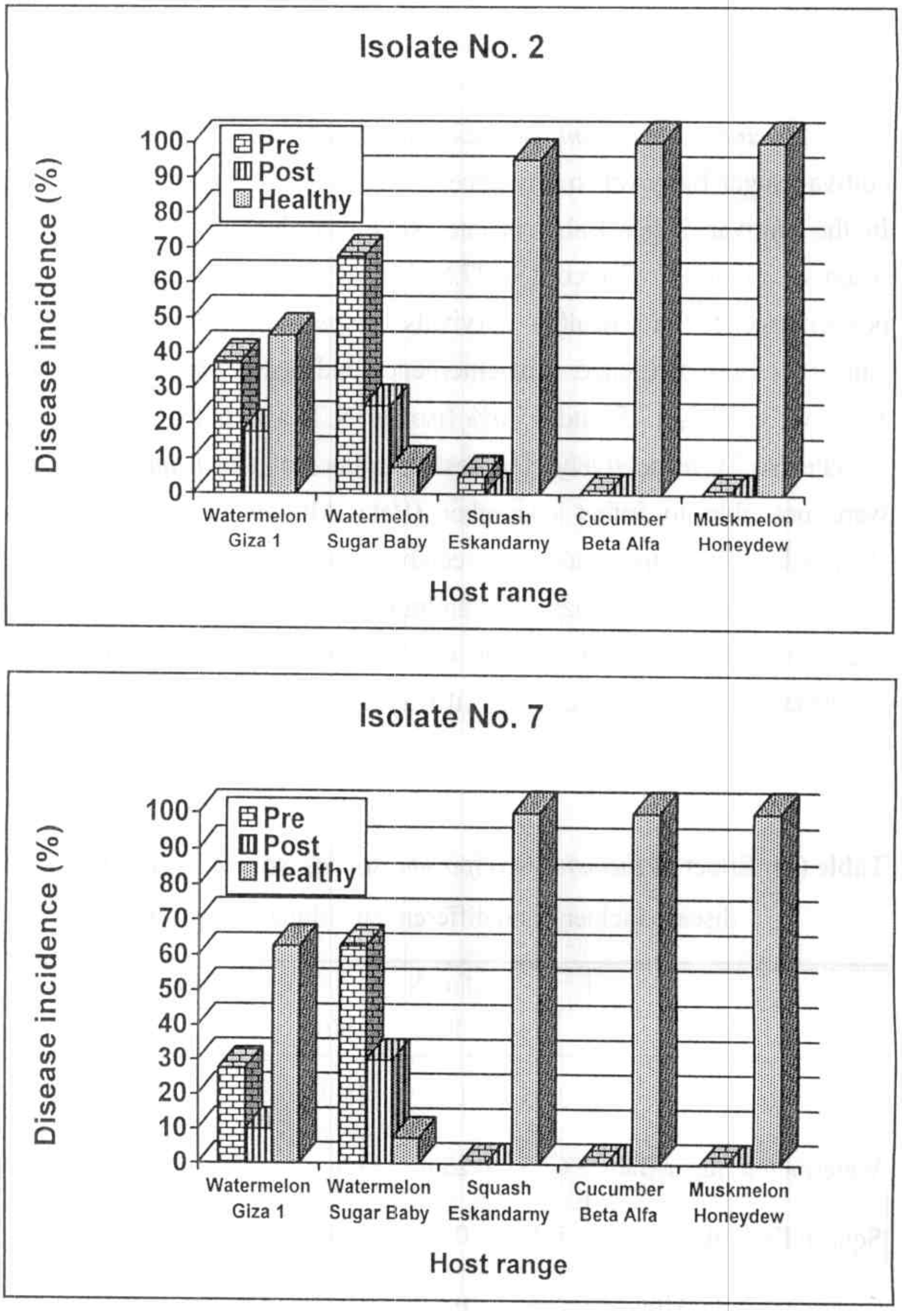


Fig. (2): Effect of *Fusarium oxysporum* isolates No. 2 & 7 on damping-off disease incidence on different cucurbitacious plants.

I.5- Varietal reaction:

Seven watermelon cultivars namely, Calhoun, Gray, Charleston Gary, Sugar Baby, Aswan hybrid, Odeum, Giza 1 and Giza 21 were tested for their reaction against infection with the aggressive *F. oxysporum* f.sp. *niveum* isolates No.2 and No.7. Data about disease assessment are shown in **Table (5)** and **Fig. (3)**. Data show clearly that the tested watermelon cultivars reacted differently against isolates No. 2 and 7 of *F. oxysporum* f.sp. *niveum*. Percentages of pre-emergence, post-emergence damping off and healthy survived seedlings were ranged between 7.5-65.0%, 5.0-25.0% and 10.0-87.5% (isolate No.2) and 10.0-62.5%, 5.0-30.0% and 7.5-85.0% (isolate No.7), respectively. Among the tested watermelon cultivars, Calhoum Gray was the most resistant since it recorded the lowest significant decrease in % pre-emergence (7.5 & 10.0%), post-emergence (5.0 & 5.0%) and highest % survival (87.5 & 85.0%) under stress of infection with isolates No.2 & No.7, respectively. On the contrary, Sugar Baby was the most susceptible one since it recorded the highest significant decrease in % pr-emergence (65.0 & 62.5%), post-emergence (25.0 & 30.0%) and produced the lowest % survival (10.0 & 7.5%) when exposed to infection with isolates No.2 & No.7, respectively. However, the cultivars Giza 21, Giza 1, Odeum, Charleston Gray and Aswan showed intermediate responses.

Table (5): Effect of infection with two isolates (No. 2 and 7) of *F. oxysporum* f.sp. *niveum* on varietal reactions.

Cultivars	% Disease incidence					
	Isolate No.2			Isolate No.7		
	Pre	Post	Healthy	Pre	Post	Healthy
Calhoum Gray	07.5c	05.0b	87.5	10.0d	05.0	85.0
Charleston Gray	25.5b	10.0b	62.5	27.5c	15.0	57.5
Sugar Baby	65.0a	25.0a	10.0	62.5a	30.0	07.5
Aswan	27.5b	12.5b	60.0	45.0b	15.0	40.0
Odeum	25.0b	10.0b	65.0	32.5c	10.0	57.5
Giza 1	27.5b	12.5b	60.0	27.5c	10.0	62.5
Giza 21	32.5b	12.5b	55.0	30.0c	05.0	65.0
L.S.D.	7.89	7.60	10.41	6.62	6.48	10.99

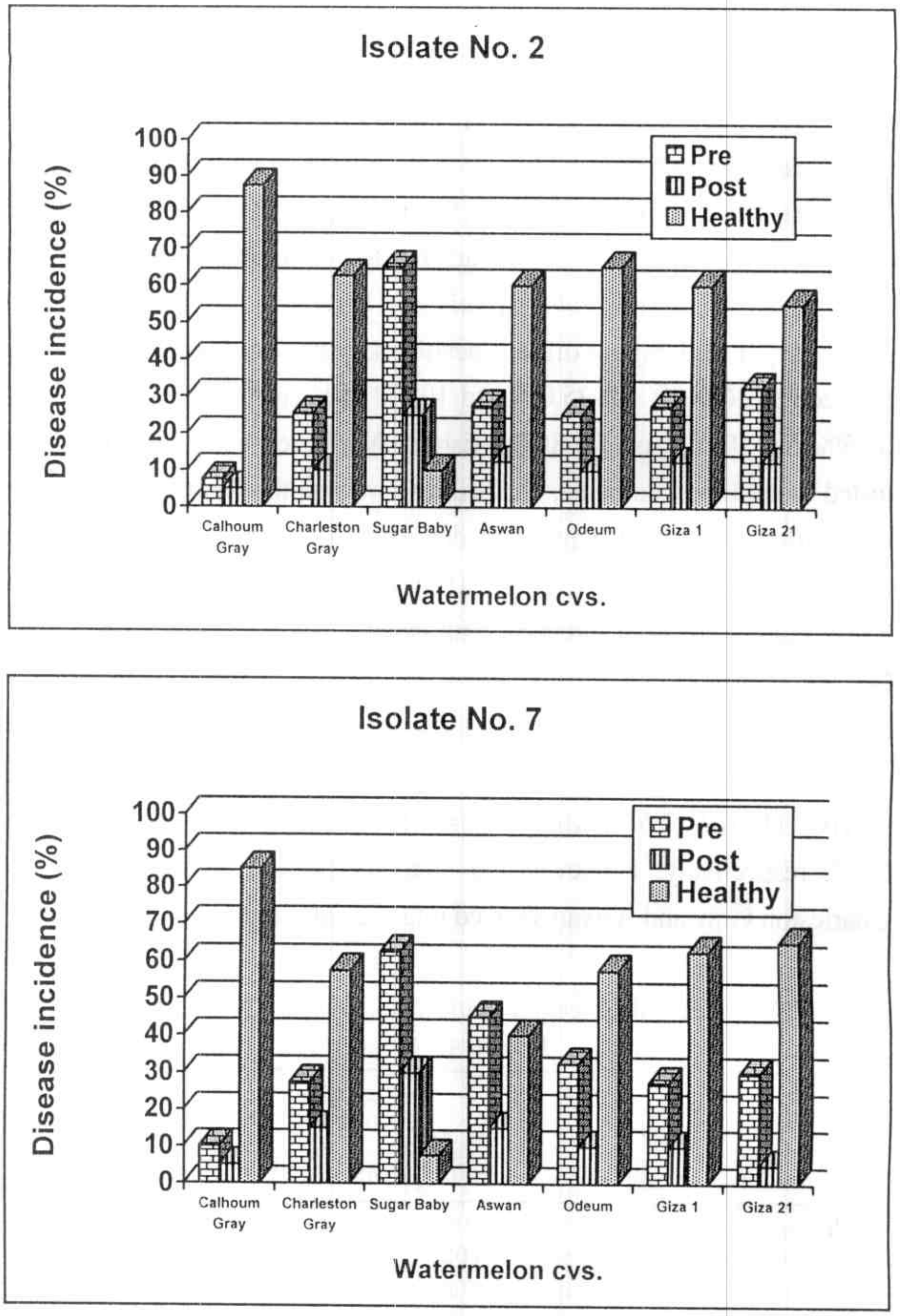


Fig. (3): Effect of infection with two isolates (No. 2 and 7) of *F. oxysporum* f.sp. *niveum* on varietal reactions.

Part II: Tissue culture experiments to induce somaclonal variation:

II.1. Production of free-contaminated cotyledons:

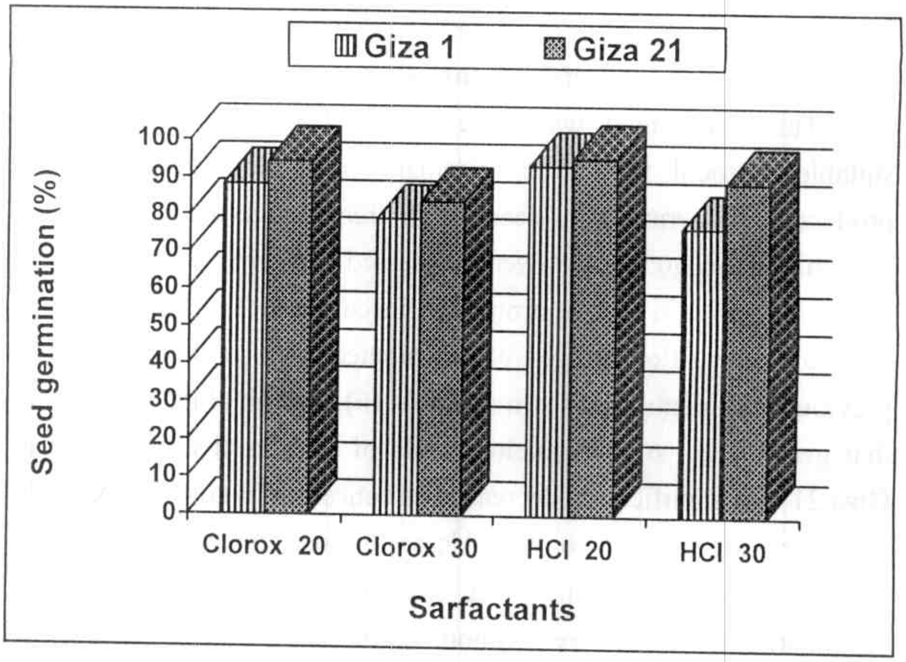
II.1.1. - Effect of seed surface disinfectants:

This experiment was done to investigate the appropriate or suitable protocol for surface sterilization of watermelon seeds to produce high germination seeds with free-contaminated cotyledons. To achieve this goal, seeds were immersed in Clorox or HCl at (20 or 30%) for 20 min and thoroughly rinsed three times in sterilized distilled water. Percentages of germinated seeds resulted from the previous treatments are shown in **Table (6)**. The obtained results show that germination of watermelon seeds of both cultivars Giza 1 and Giza 21 was significantly decreased as concentration of Clorox or HCl was increased from 20 to 30%. Using the low concentration (20.0%) of Clorox or HCl caused in the highest percentages of seed germination in Giza 21 cv., without significant differences in between whereas HCl was significantly better than Clorox in Giza 1 cv. On the contrary, using Clorox or HCl at 30% showed no significant differences in germination of Giza 1 seeds whereas HCl was significantly better than Clorox in case of Giza 21 cv. The cotyledons separated from watermelon seeds previously disinfected with Clorox or HCl at either 20.0 or 30.0% were 100.0% contaminated with bacteria when cultured on the MS medium.

Table (6): Effect of Clorox and HCl at 20 & 30% concentrations as seed surface disinfectants on % seeds germination and contaminated *cotyledons of watermelon Giza 1 and Giza 21 cultivars.

Disinfectant		% Germination		% Contamination	
Surfactants	Conc. (%)	Giza 1	Giza 21	Giza 1	Giza 21
Clorox	20	88.3	94.3	100.0	100.0
	30	79.0	83.7	100.0	100.0
HCl	20	93.3	95.3	100.0	100.0
	30	76.7	89.0	100.0	100.0
L.S.D at 0.05		3.90	2.68		

* Cotyledons were harvested from germinated seeds and cultured on MS medium.



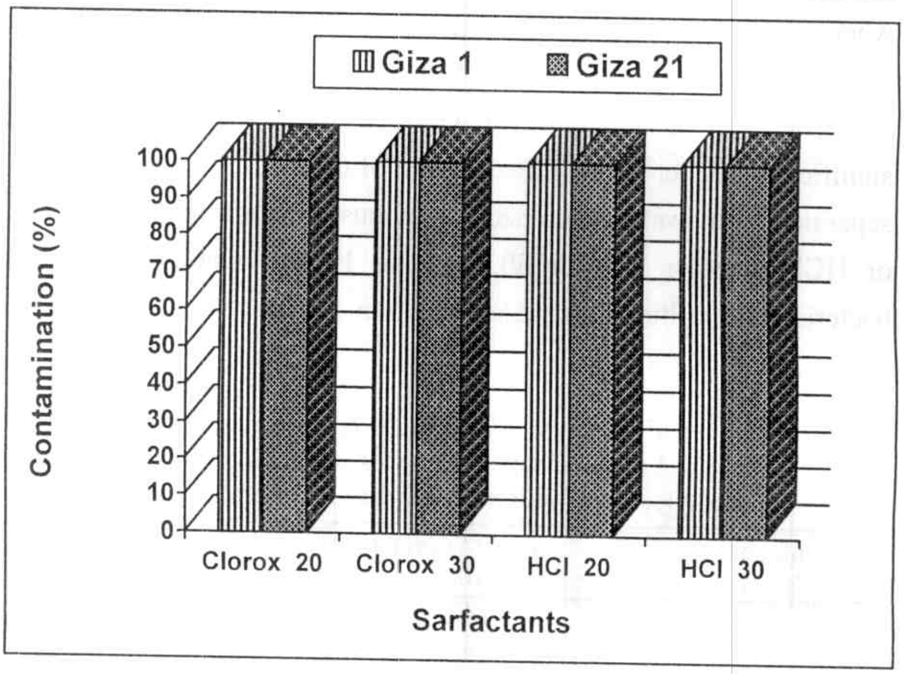


Fig (4): Effect of Clorox and HCl at 20 & 30% concentrations as seed surface disinfestants on % seeds germination and contaminated *cotyledons of watermelon cvs. Giza 1 and Giza 21.

II.1.2. Effect of dual seed and cotyledon surface disinfectants:

In this study, seeds of watermelon Giza 1 and Giza 21 cvs. were surface sterilized and allowed to germinate as mentioned under materials and methods. Then cotyledons were harvested from the germinated seeds and surface sterilized similarly for another 20 min. in 0.0 (control), 20.0, 25.0 or 30.0% of Clorox or HCl. Percentages of survived cotyledons and contaminated cotyledons as affected by these applications are recorded. Data in **Table (7)** and **Fig. (5)** reveal that the watermelon cotyledons in control treatment (non-sterilized) showed the highest percentages of survivals and contamination (100.0%) particularly in Giza 1 cv. In sterilized cotyledons, percentages of survivals and contamination were reduced significantly by increasing concentration of Clorox or HCl from 20.0 to 25.0 or 30.0% compared with control. Regardless watermelon cultivar, cotyledons sterilized in 20.0 or 30.0% HCl or 30.0% Clorox showed the lowest percentage of contamination (0.0-28.3%). Using 20.0 or 30.0% HCl seems to be better in decreasing contamination (0.0-6.7%) than 30.0% Clorox (13.3-28.3%). The obtained results showed that re-sterilization of cotyledons that were harvested from watermelon seeds previously sterilized in 20 or 30% Clorox or HCl was more effective in removing contamination from seed cotyledons of Giza 1 than those of Giza 21 cvs.

Table (7): Effect of dual surface sterilization of watermelon seeds and cotyledons on % survived and contaminated *cotyledons of watermelon cvs. Giza 1 and Giza 21.

Sterilization treatment		% Survived cotyledons		% Contaminated cotyledons	
Seed sterilization	Cotyledon sterilization	Giza 1	Giza 21	Giza 1	Giza 21
Clorox 20%	Control	100.0	100.0	100.0	100.0
	20%	90.0	96.7	68.3	78.3
	25%	86.7	90.0	70.0	75.0
	30%	66.7	75.0	23.3	43.3
Clorox 30%	Control	100.0	100.0	100.0	100.0
	20%	70.0	75.0	61.7	76.7
	25%	58.3	63.3	33.3	63.3
	30%	50.0	55.0	13.3	28.3
HCl 20%	Control	100.0	100.0	96.7	91.7
	20%	83.3	86.7	33.3	45.0
	25%	80.0	83.3	6.7	8.3
	30%	76.7	83.3	1.7	5.0
HCl 30%	Control	100.0	100.0	91.7	81.7
	20%	76.7	80.0	20.0	33.3
	25%	66.7	66.7	6.7	10.0
	30%	58.3	60.0	0.0	6.7
L.S.D at 0.05		9.27	7.15	5.80	7.96

* Cotyledons were harvested from germinated seeds and cultured on MS medium.

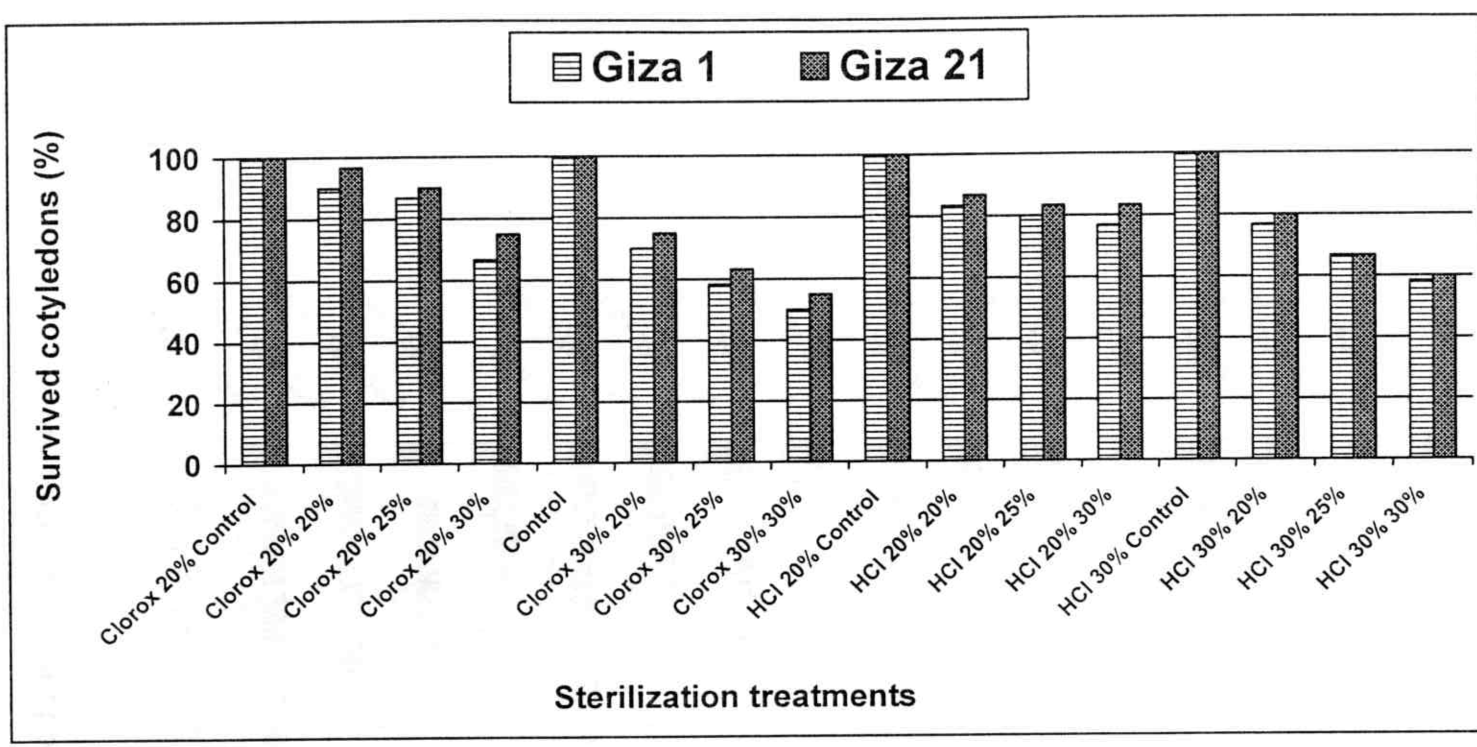


Fig. (5-A): Effect of dual surface sterilization of watermelon seeds and cotyledons on % survived cotyledons of watermelon Giza 1 and Giza 21 cultivars.

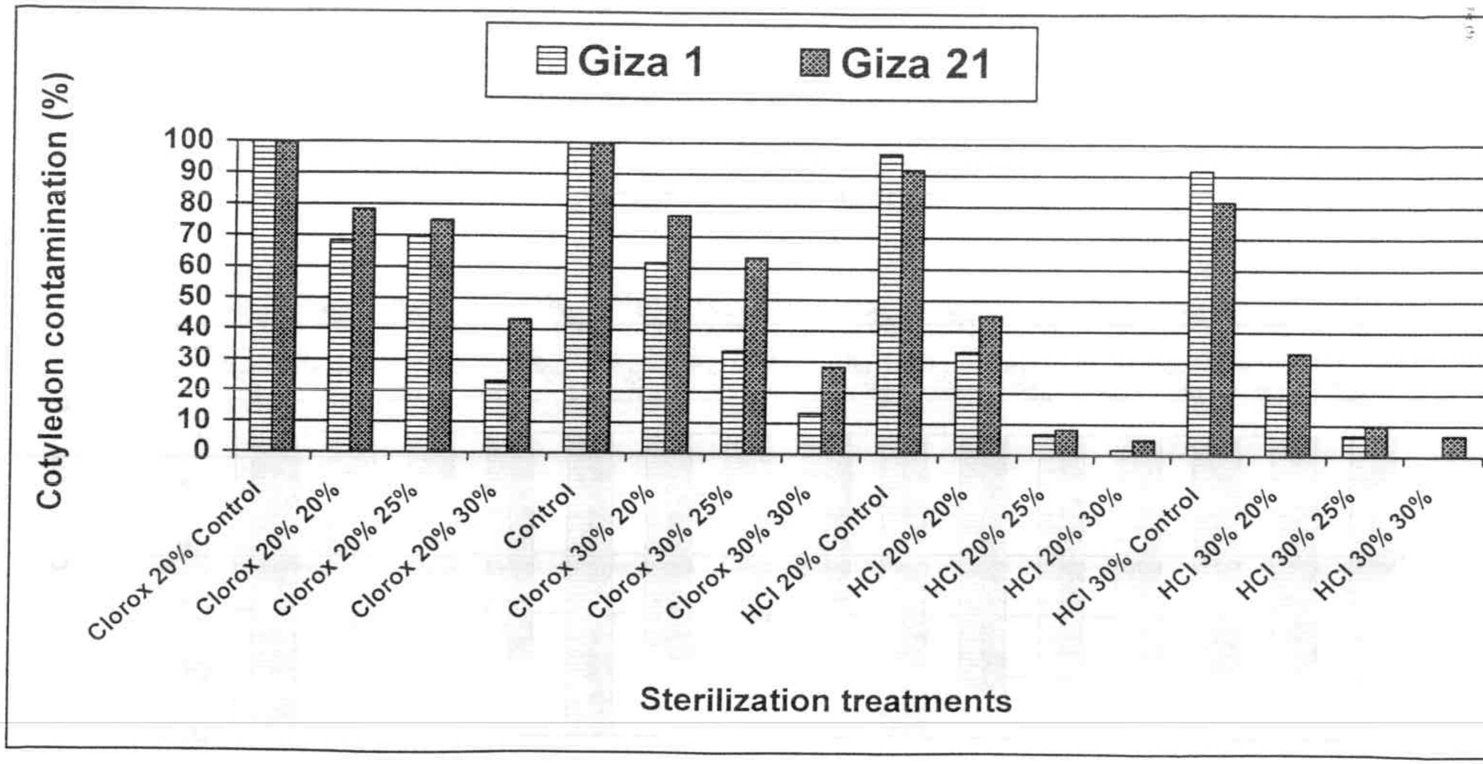


Fig. (5-B): Effect of dual surface sterilization of watermelon seeds and cotyledons on % contaminated cotyledons of watermelon Giza 1 and Giza 21 cultivars.

II.2. Production of the diploid adventitious shoots of two watermelon cultivars via direct organogenesis:

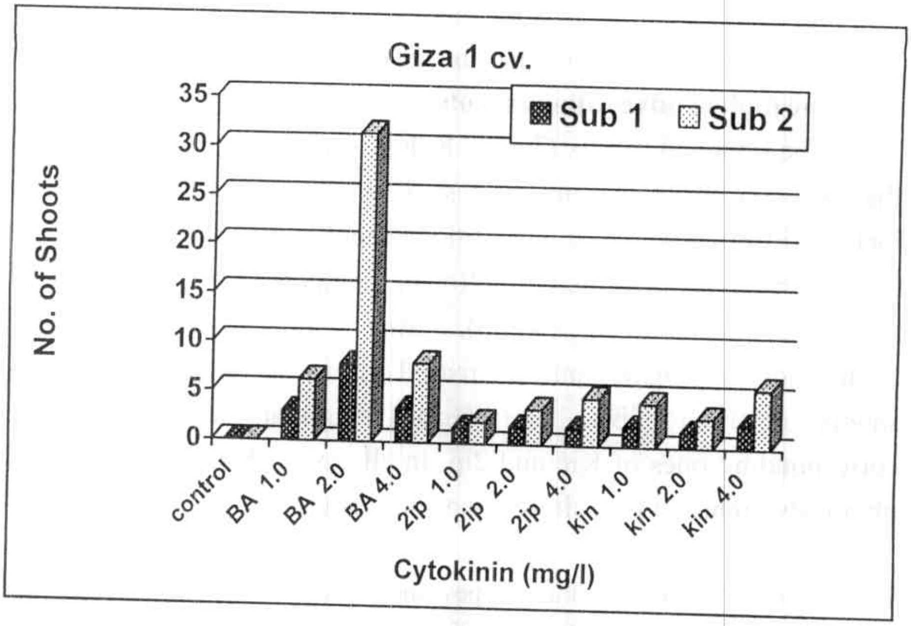
II.2.1. Effect of cytokinin type and concentrations on formation of the diploid adventitious shoots:

The obtained results **(Table, 8; Fig. 6 & 7** and **Photo, 2)** reveal that all concentrations of the tested cytokinin types significantly increased formation of the adventitious shoots estimated as number of shoots in both watermelon cultivars compared with the control treatment (without cytokinin). Any concentration of BA (Benzyladinine) significantly increased formation of the adventitious shoots in both Giza1 and Giza21 cultivars more than the corresponding ones of Kin and 2ip. In all cases, shoot formation was obviously higher in subculture 2 than subculture 1.

Table (8): Effect of cytokinin types and concentrations on adventitious shoot formation and hyperhydericity of watermelon cvs. Giza 1 and Giza 21 during two subcultures.

Cytokinin & concentrations (mg/l)		Shoot formation (number)				Hyperhydericity			
		Giza1		Giza21		Giza1		Giza21	
		Sub 1	Sub 2	Sub 1	Sub 2	Sub 1	Sub 2	Sub 1	Sub 2
BA	1.0	3.0	6.2	2.4	5.4	1.7	3.0	2.0	3.3
	2.0	7.8	31.4	4.4	15.0	2.7	3.7	3.3	4.3
	4.0	3.4	8.0	1.8	4.4	3.0	5.0	3.7	5.0
2iP	1.0	1.6	2.2	2.0	2.8	1.3	2.7	1.3	2.7
	2.0	1.8	3.6	1.0	3.6	1.7	3.7	2.0	3.7
	4.0	1.8	4.8	1.4	3.0	2.0	4.3	2.7	4.3
kin	1.0	2.0	4.4	2.2	3.8	1.3	2.0	2.0	2.7
	2.0	1.6	3.0	1.8	4.4	1.7	4.0	2.3	3.3
	4.0	2.4	6.0	2.2	5.0	2.0	4.3	3.0	4.3
Control		0.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0
LSD at 0.05		0.80	1.02	0.62	1.33	0.76	0.73	1.07	0.82

Some produced explants became vitrified and dead (hyperhydericity phenomenon). Increasing concentrations of any tested cytokinin significantly increased the degree of hyperhydericity. Accordingly, media containing the highest cytokinin conc. (4 mg/liter) induced the highest incidence of hyperhydericity. In both watermelon cultivars, subculture No.2 on media contained BA showed the highest degree of hyperhydericity (5.0) followed by kin and 2ip (4.3).



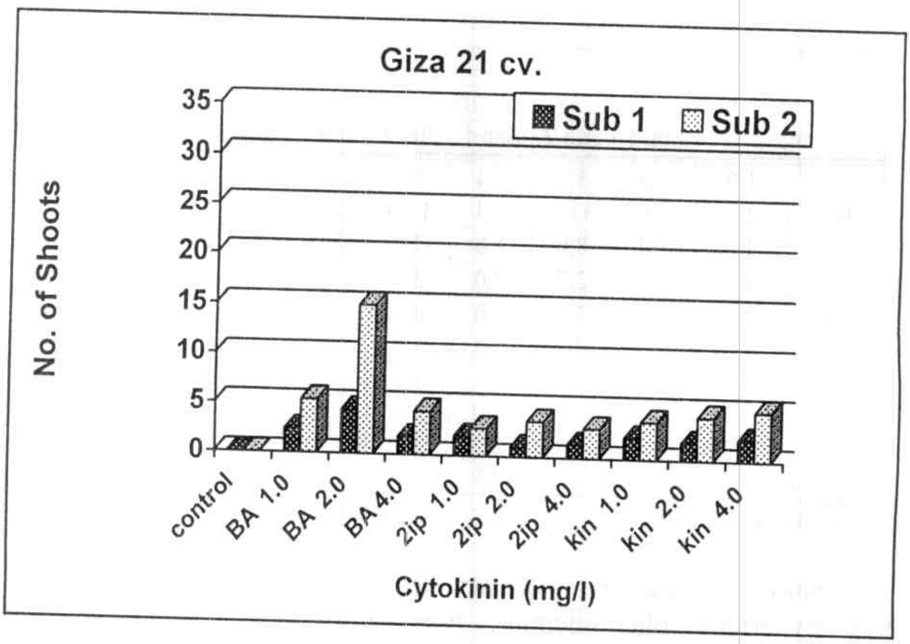
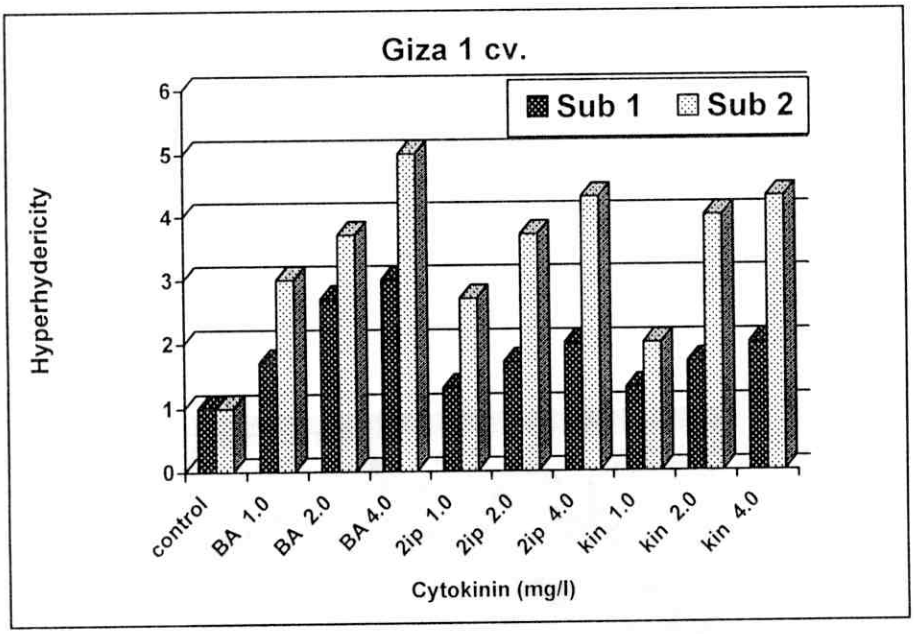


Fig (6): Effect of cytokinin types and concentrations on the number of adventitious shoots of watermelon Giza 1 and Giza 21 cvs. during two subcultures.



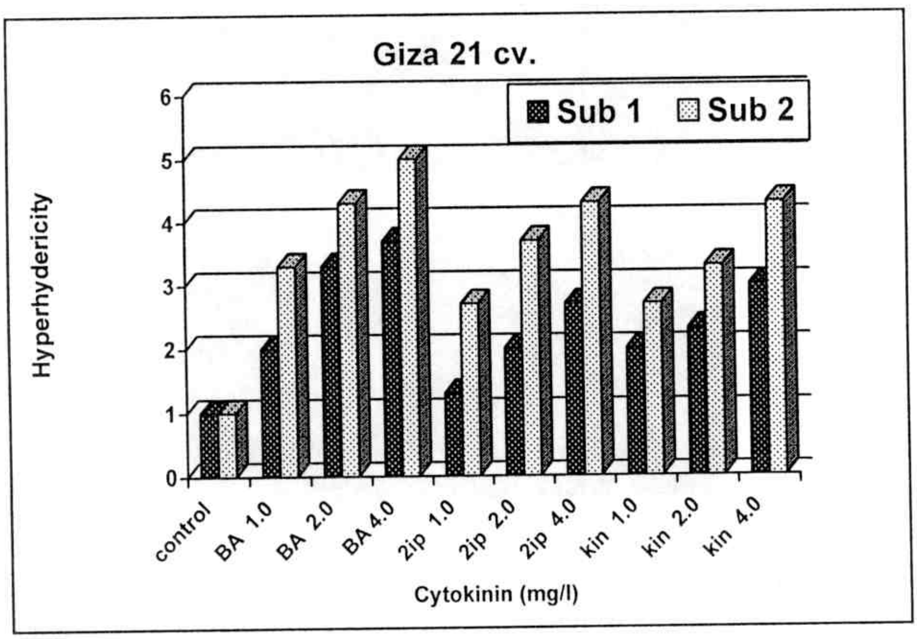


Fig (7): Effect of cytokinin types and concentrations on hyperhydericity of watermelon Giza 1 and Giza 21 cvs. during two subcultures.

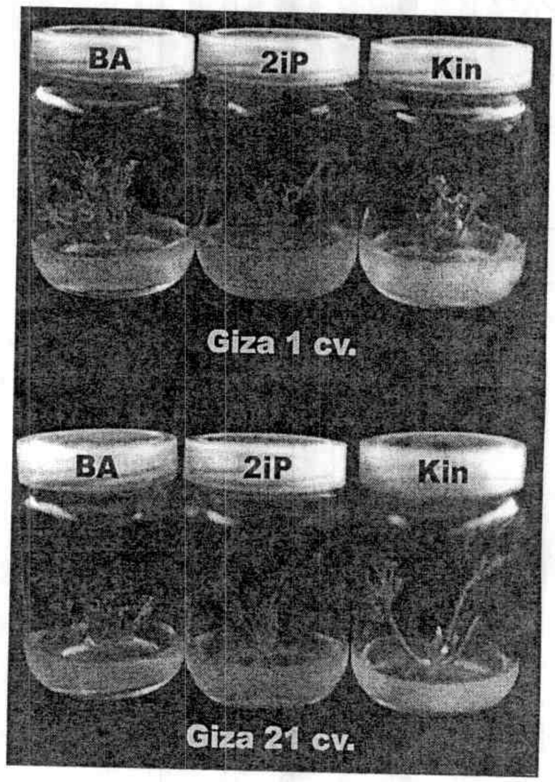


Photo (2): Effect of adding BA, 2iP and kin (at 2 mg/L) to culture medium on number of adventitious shoots produced on watermelon cvs. Giza 1 and Giza 21.

II.2.2. Effect of different concentrations and combinations of IBA and BA on regeneration of the diploid watermelon plantlets:

Data in **Table (9)** and **Fig. (8)** clearly reveal that number of branches/explant (NBE) of 2 watermelon cultivars Giza 1 and Giza 21 was affected significantly by the tested concentrations of BA (cytokinin) and IBA (auxin). In the absence of BA, adding IBA to the MS-medium had no significant effect on the NBE compared with the control treatment. However, the NBE was proportionally increased with increasing concentrations of both IBA and BA. Among the tested IBA+BA combinations, IBA (0.0 or 0.1 mg/liter) + BA (1.0 mg/liter) produced the highest NBE. This trend was true in both watermelon cultivars Giza1 and Giza21 either during subculture 1 or 2.

Table (9): Effect of IBA and BA at different concentrations on the average number of branches/explant of 2 watermelon cvs. during two successive subcultures.

Treatments		Average number of branches/explant			
		Giza 1 cv.		Giza 21 cv.	
BA (mg/l)	IBA (mg/l)	Subculture 1	Subculture 2	Subculture 1	Subculture 2
0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.1	0.0	0.3	0.0	0.0
0.0	0.5	0.0	0.7	0.7	0.7
0.0	1.0	0.7	0.0	0.0	0.0
0.1	0.0	2.3	9.0	1.3	13.3
0.1	0.1	2.7	9.7	1.3	14.0
0.1	0.5	2.3	8.7	1.7	10.0
0.1	1.0	2.0	5.3	2.3	7.0
0.5	0.0	4.0	13.7	4.0	17.7
0.5	0.1	4.0	13.0	2.7	14.7
0.5	0.5	4.3	12.7	2.0	14.3
0.5	1.0	3.7	3.7	3.3	4.7
1.0	0.0	7.7	23.0	8.0	20.7
1.0	0.1	7.3	24.7	7.7	21.7
1.0	0.5	5.0	16.3	2.3	13.7
1.0	1.0	3.0	1.0	1.3	0.0
LSD at 0.05		1.58	1.53	1.00	1.95

Fig. (8)

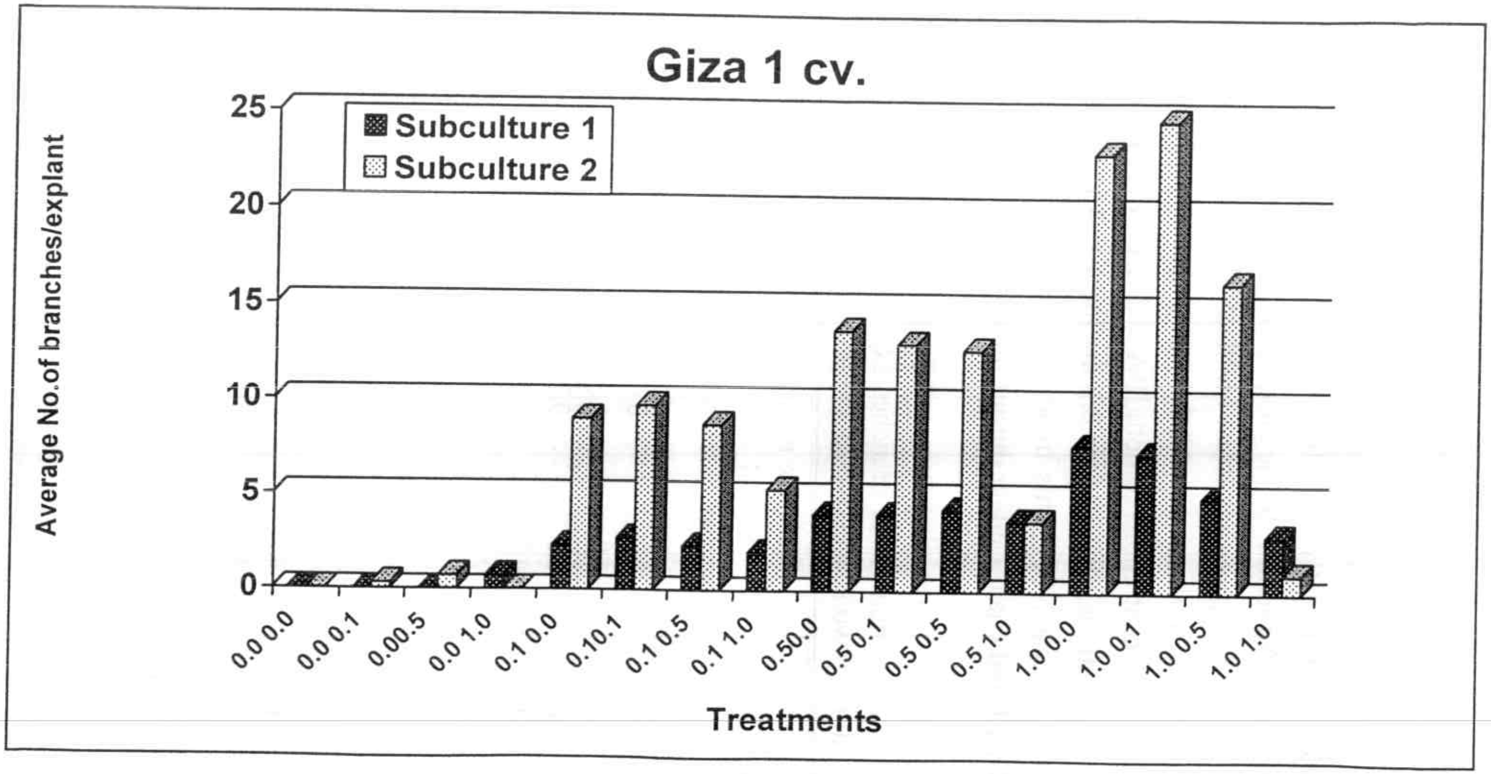


Fig. (8-A): Effect of IBA and BA at different concentrations on the average number of branches/explant of watermelon Giza 1 cv. during two successive subcultures.

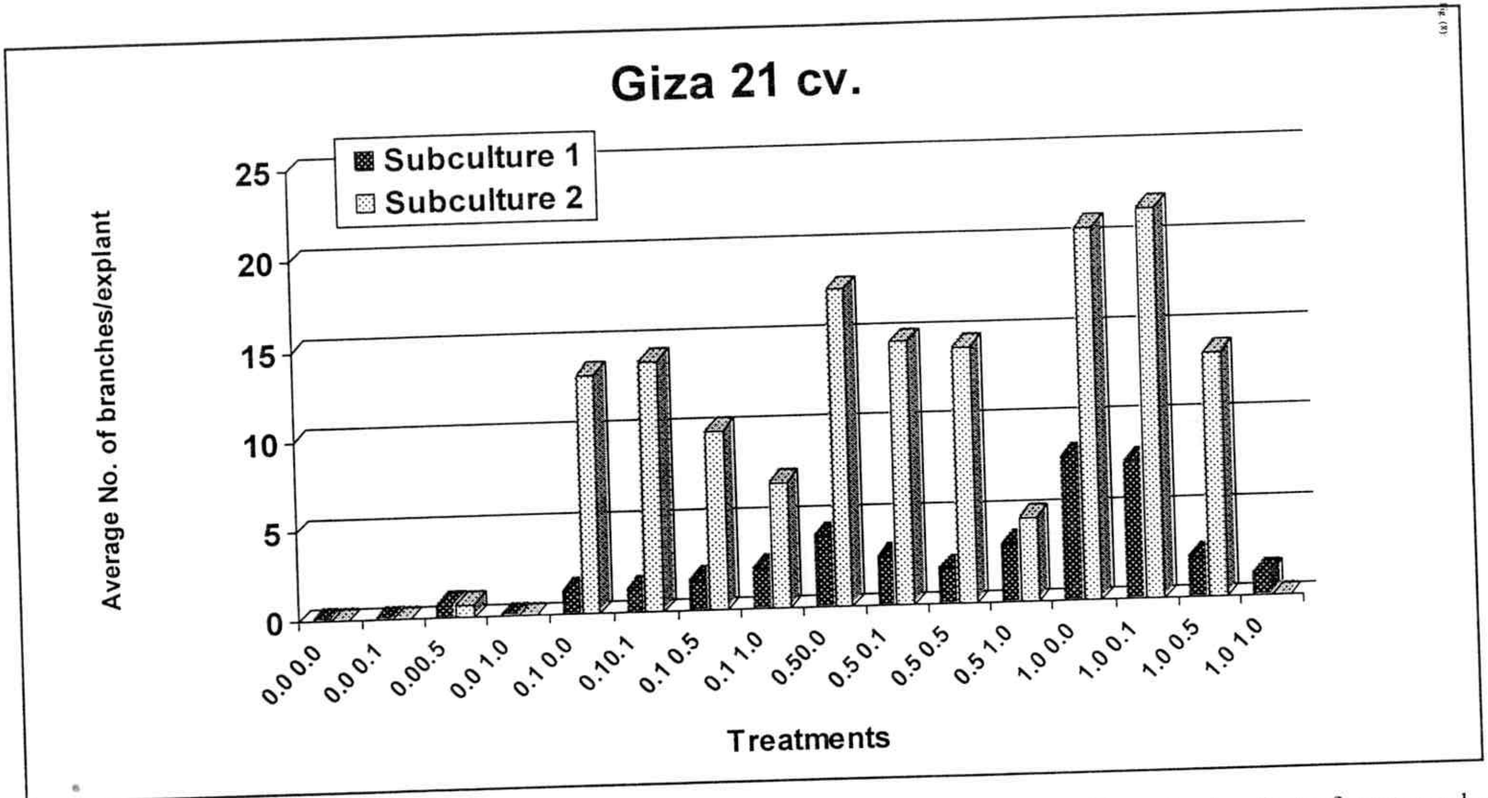


Fig. (8-B): Effect of IBA and BA at different concentrations on the average number of branches/explant of watermelon Giza 21 cv. during two successive subcultures.

The number of nodes/plantlet (NNP) also was significantly affected by the tested treatments **(Table, 10)** and **Fig. (9)**. In the absence of BA, increasing concentrations of IBA from 0.0 to 1.0 mg/liter significantly increased the shoot proliferation particularly during subculture 2. However, in the presence of IBA, additional increase in the NNP was obtained by increasing BA concentrations up to 0.5 mg/liter. In general, the MS-medium contained only BA at the rate of 1.0 mg/liter or 0.1 mg/liter BA + 0.5 mg/liter IBA produced the highest NNP during subculture No. 1 of Giza1 (36.0) and Giza21 (28), respectively. While, IBA at 0.1 mg/liter + IBA at 1.0 mg/liter produced the highest NNP during subculture2 of Giza1 (182.7) and Giza 21 (175.3).

Table (10): Effect of IBA and BA at different concentrations on the average number of nodes/plantlet of 2 watermelon cvs. during two subcultures.

Treatments		Average number of nodes/plantlet			
		Giza 1 cv.		Giza 21 cv.	
BA (mg/l)	IBA (mg/l)	**Subculture 1**	**Subculture 2**	**Subculture 1**	**Subculture 2**
0.0	0.0	12.0	67.7	10.7	60.0
0.0	0.1	12.7	72.7	13.3	68.0
0.0	0.5	15.7	110.0	14.7	105.3
0.0	1.0	21.7	171.0	21.3	156.7
0.1	0.0	11.0	62.7	12.7	65.7
0.1	0.1	14.7	81.7	11.3	61.7
0.1	0.5	36.0	114.3	17.3	132.3
0.1	1.0	26.0	182.7	25.3	175.3
0.5	0.0	15.0	74.7	18.3	90.3
0.5	0.1	20.3	92.0	16.0	84.3
0.5	0.5	29.3	106.3	19.3	144.7
0.5	1.0	22.0	164.0	23.7	159.3
1.0	0.0	25.7	115.7	28.0	104.3
1.0	0.1	34.3	99.0	24.3	98.0
1.0	0.5	17.3	93.0	15.3	109.0
1.0	1.0	19.7	102.0	9.7	97.7
LSD at 0.05 for		3.14	7.69	2.23	10.20

Fig-9

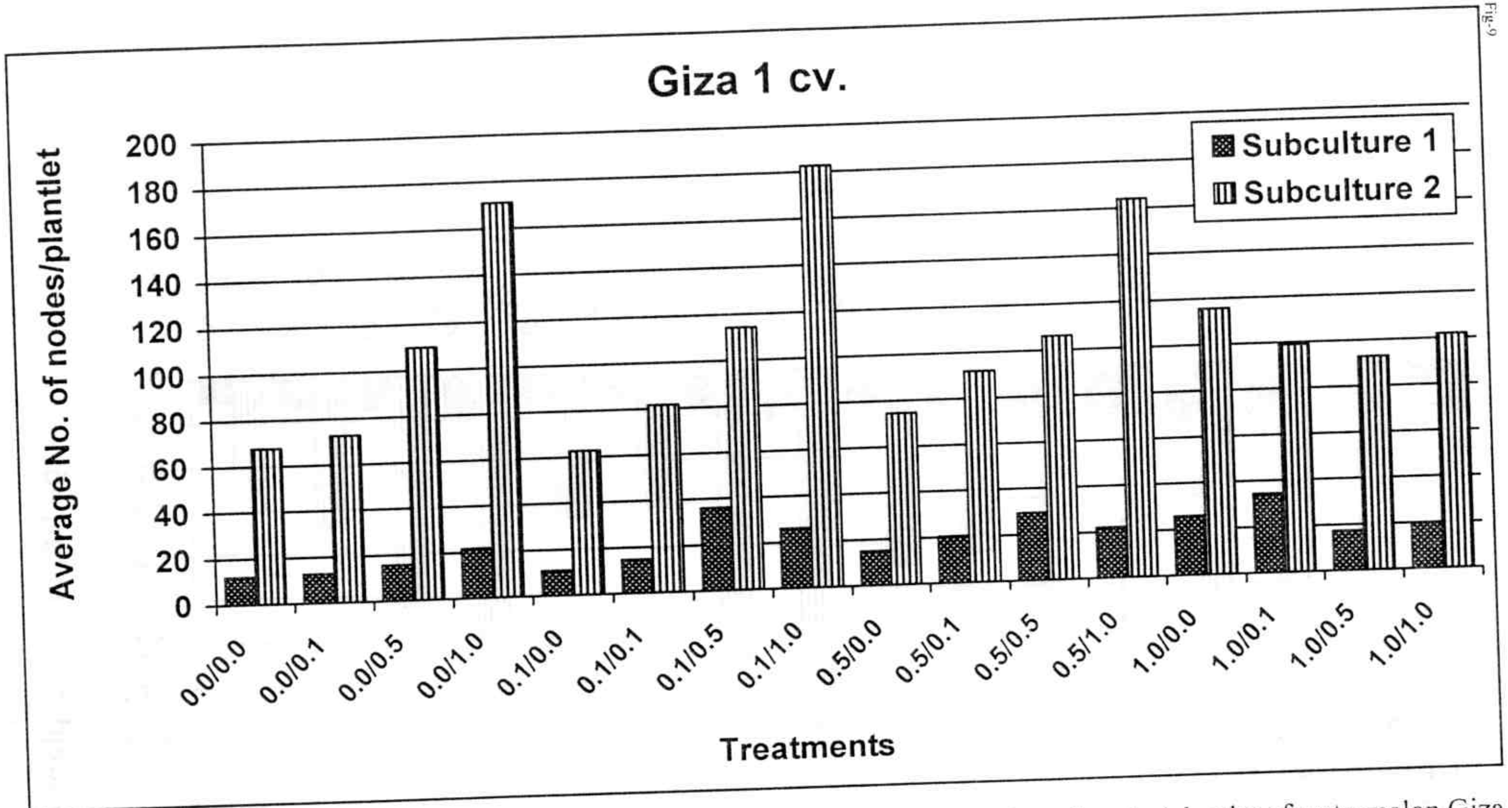


Fig. (9-A): Effect of IBA and BA at different concentrations on the average number of nodes/plantlet of watermelon Giza 1 cv. during two subcultures.

Fig-9

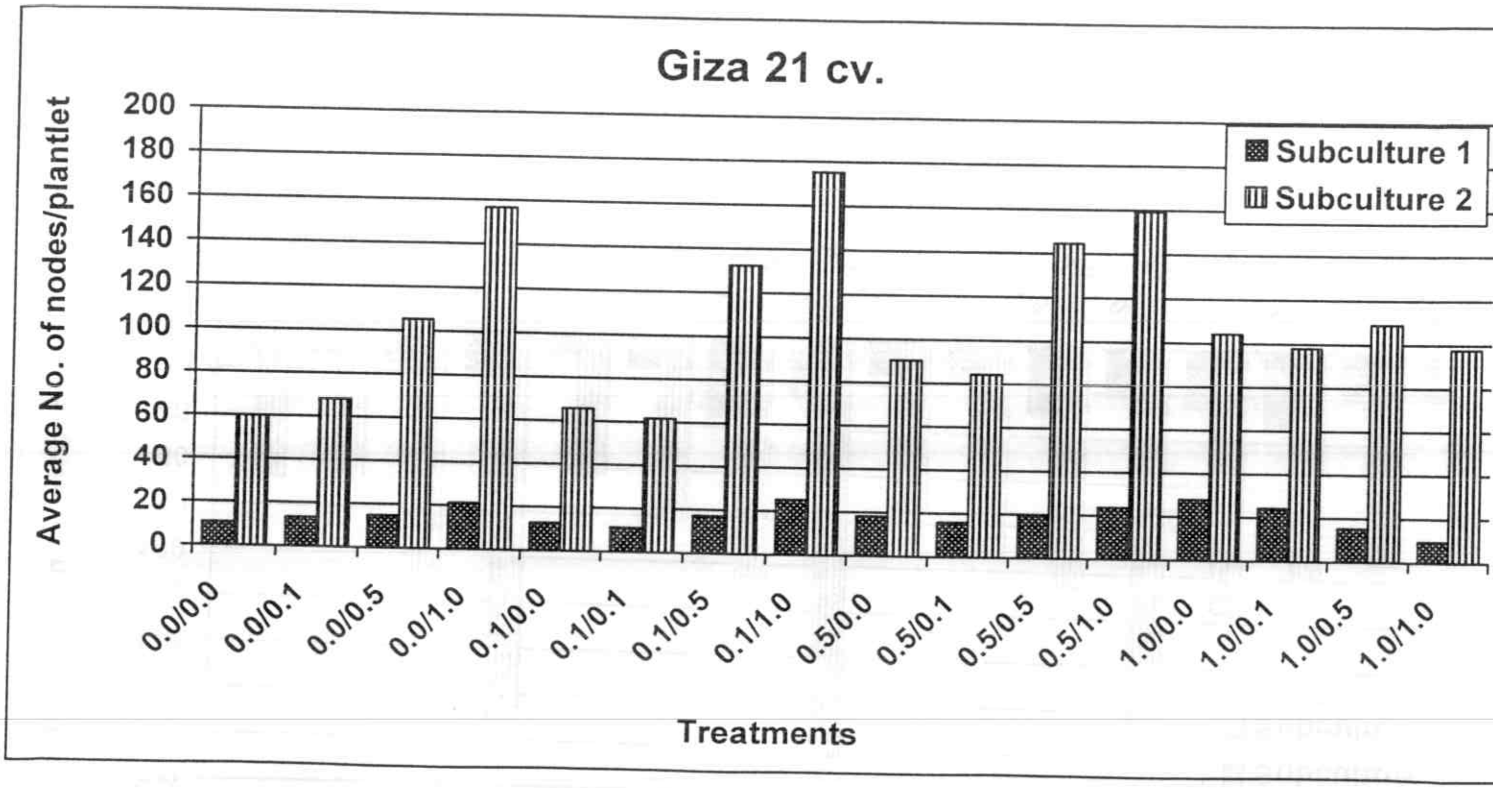


Fig. (9-B): Effect of IBA and BA at different concentrations on the average number of nodes/plantlet of watermelon Giza 21 cv. during two subcultures.

The number of leaves//plantlet (NLP) was also affected similarly **(Table, 11)** and **Fig. (10)** with few exceptions. The treatment of BA (1.0 mg/liter) alone or combined with IBA (0.5 mg/liter) produced the highest NLP in Giza21 (30.0) and Giza1 (37.3), respectively during subculture1. While, the combined treatment consisted of BA (0.1 mg/liter) + IBA (1.0 mg/liter) was the best during subculture2 for increasing NLP *i.e.* 176.7 & 184.0 in both watermelon cultivars, respectively.

Table (11): Effect of IBA and BA at different concentrations on the average number of leaves/plantlet of 2 watermelon cvs. during two subcultures.

Treatments		Average number of nodes/plantlet			
		Giza 1 cv.		Giza 21 cv.	
BA (mg/l)	IBA (mg/l)	Subculture 1	Subculture 2	Subculture 1	Subculture 2
0.0	0.0	13.3	69.0	12.0	62.0
0.0	0.1	13.7	73.3	14.0	70.0
0.0	0.5	17.3	111.7	15.7	106.7
0.0	1.0	23.3	172.3	24.0	157.7
0.1	0.0	12.7	64.0	13.7	66.7
0.1	0.1	15.7	82.3	13.7	63.3
0.1	0.5	30.3	116.0	18.0	133.7
0.1	1.0	27.7	184.0	26.3	176.7
0.5	0.0	16.0	76.0	19.3	91.7
0.5	0.1	21.7	94.0	10.7	85.7
0.5	0.5	19.0	108.3	20.6	147.7
0.5	1.0	22.7	164.7	25.0	163.3
1.0	0.0	27.3	117.7	30.0	106.0
1.0	0.1	36.3	100.3	25.3	98.3
1.0	0.5	37.3	94.3	17.0	110.7
1.0	1.0	21.3	103.3	10.6	164.7
LSD at 0.05 for		3.59	7.24	2.47	10.34

Fig 10

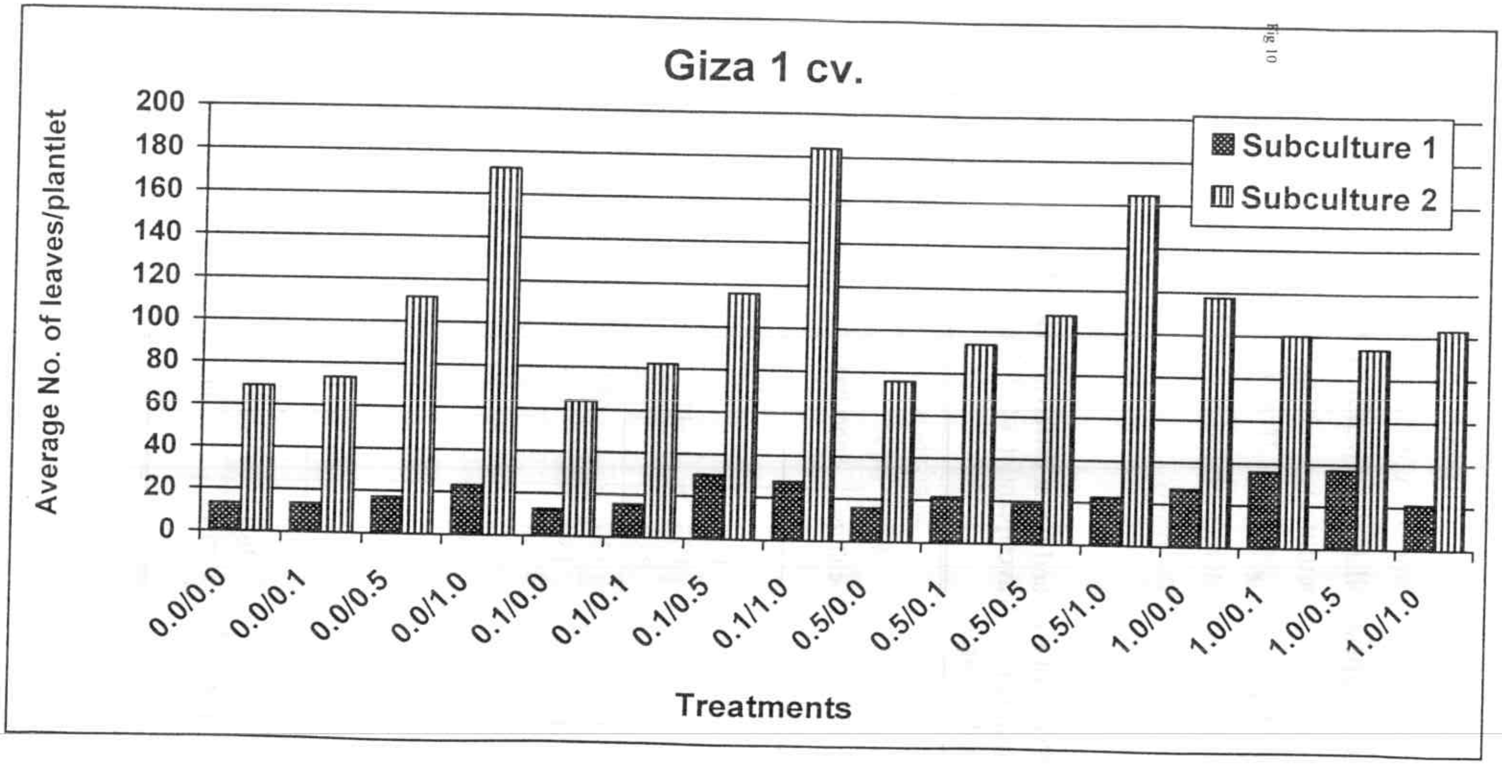


Fig (10-A): Effect of IBA and BA at different concentrations on the average number of leaves/plantlet of watermelon Giza 1 cv. during two subcultures.

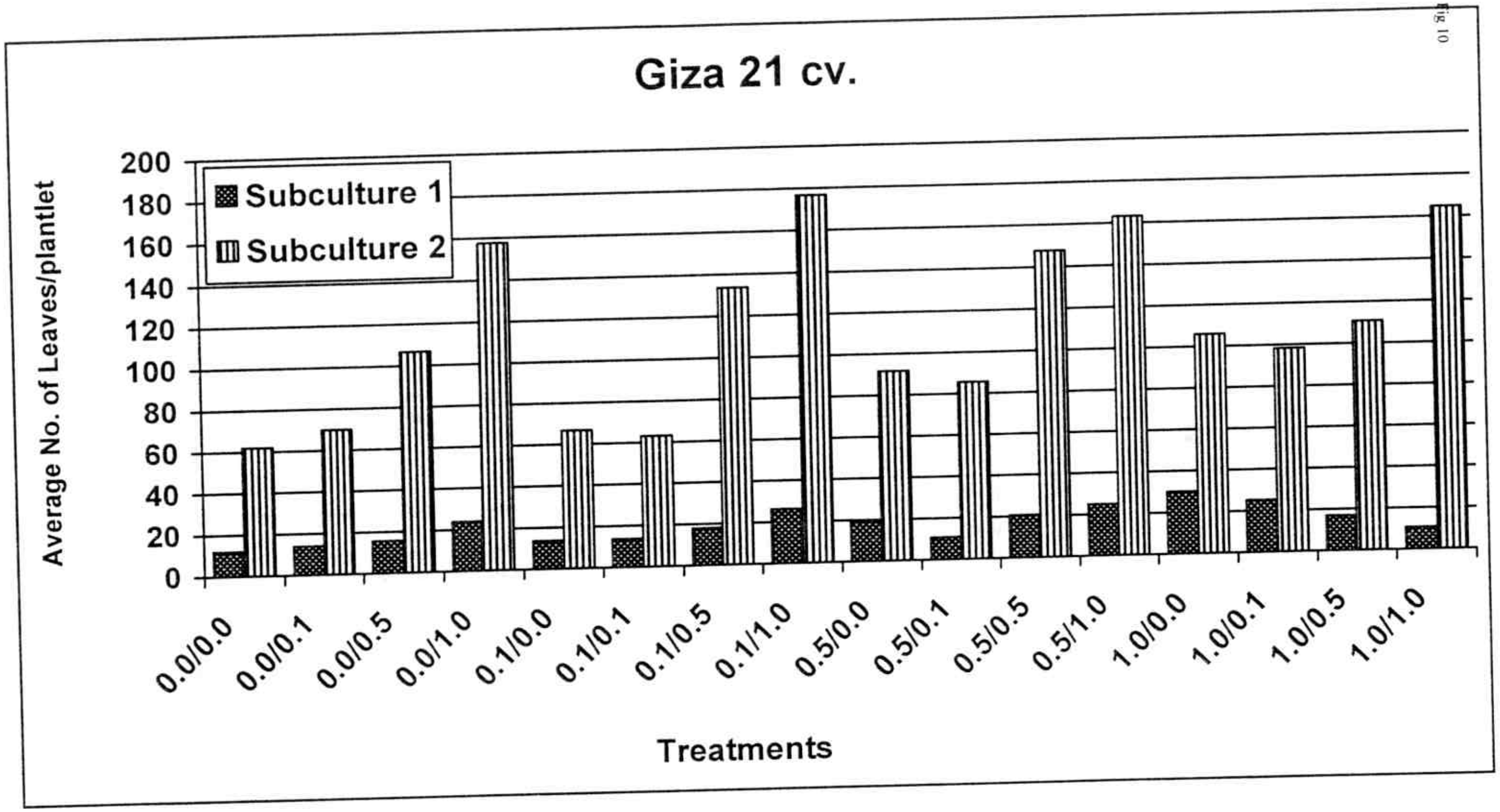


Fig (10-B): Effect of IBA and BA at different concentrations on the average number of leaves/plantlet of watermelon Giza 21 cv. during two subcultures.

As for plantlet length (PL), data in **Table (12)** and **Fig. (11)** show that using BA at a concentration more than 0.1 mg/liter caused significant decrease in PL of both watermelon Giza 1 and Giza 21 cultivars. On the contrary, BA (1.0 mg/liter) + IBA (1.0 mg/liter) induced the highest significant increase in the PL of both watermelon cultivars compared with the control treatment (no BA no IBA).

Table (12): Effect of IBA and BA at different concentrations on the average length of adventitious shoots of 2 watermelon cvs. during two subcultures.

Treatments		Average length of plantlet (cm)			
		Giza 1 cv.		Giza 21 cv.	
BA (mg/l)	IBA (mg/l)	Subculture 1	Subculture 2	Subculture 1	Subculture 2
0.0	0.0	11.3	9.0	12.0	10.7
0.0	0.1	12.7	12.0	13.3	13.3
0.0	0.5	11.0	11.3	13.7	13.3
0.0	1.0	13.7	15.3	14.3	17.3
0.1	0.0	14.0	13.7	14.7	13.7
0.1	0.1	11.7	12.3	12.3	13.0
0.1	0.5	9.0	10.7	10.3	11.3
0.1	1.0	18.3	19.0	19.7	20.0
0.5	0.0	8.0	5.0	8.7	6.7
0.5	0.1	5.0	3.7	5.3	4.3
0.5	0.5	9.0	8.3	10.7	9.7
0.5	1.0	8.7	12.3	11.7	13.0
1.0	0.0	3.7	0.7	4.0	1.7
1.0	0.1	6.7	3.0	7.0	2.3
1.0	0.5	5.7	3.7	7.0	4.3
1.0	1.0	1.3	0.8	2.3	1.0
LSD at 0.05 for		2.32	1.44	1.69	2.05

Fig 11

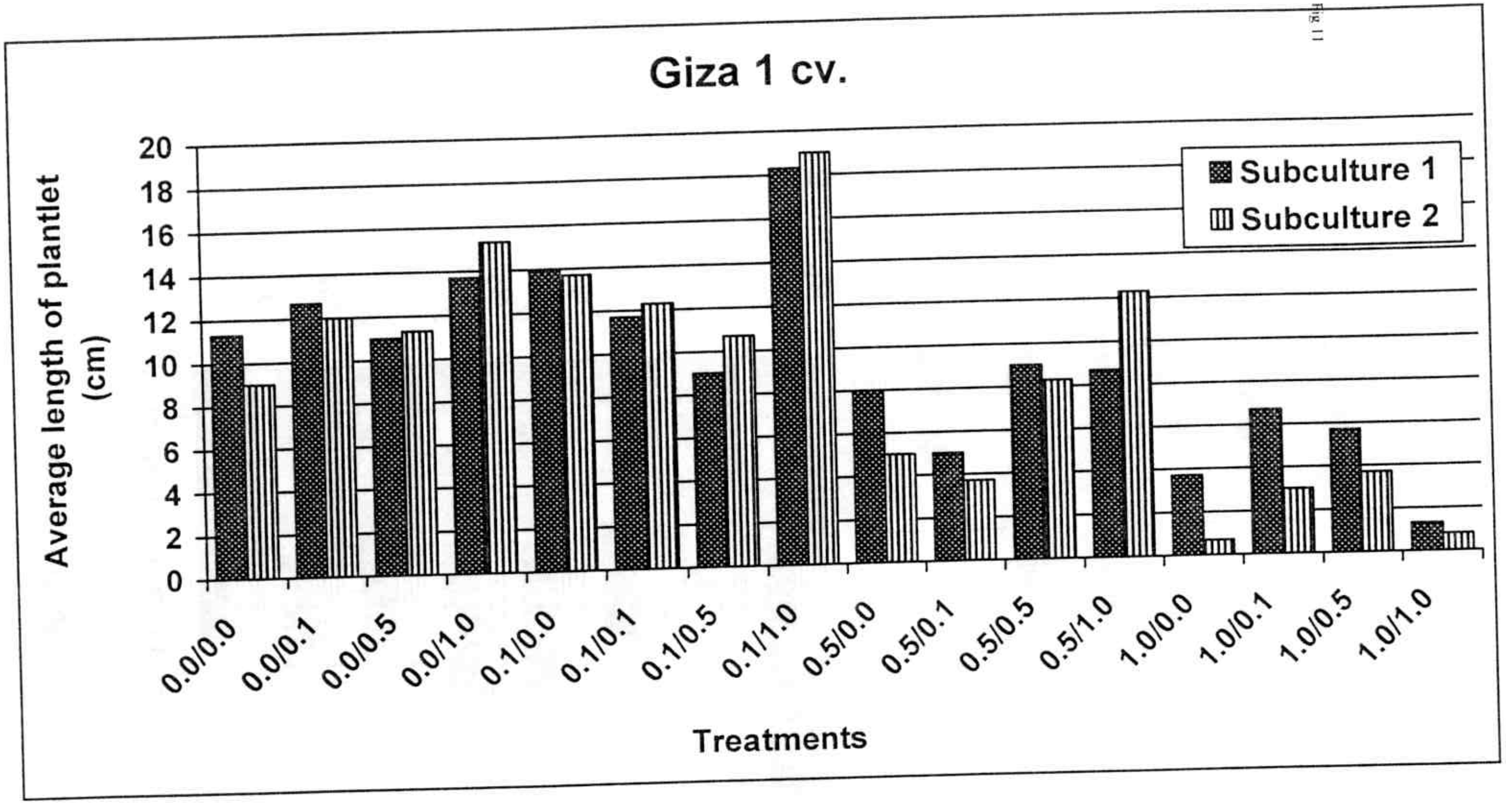


Fig (11-A): Effect of IBA and BA at different concentrations on the average length of adventitious shoots of watermelon Giza 1 cv. during two subcultures.

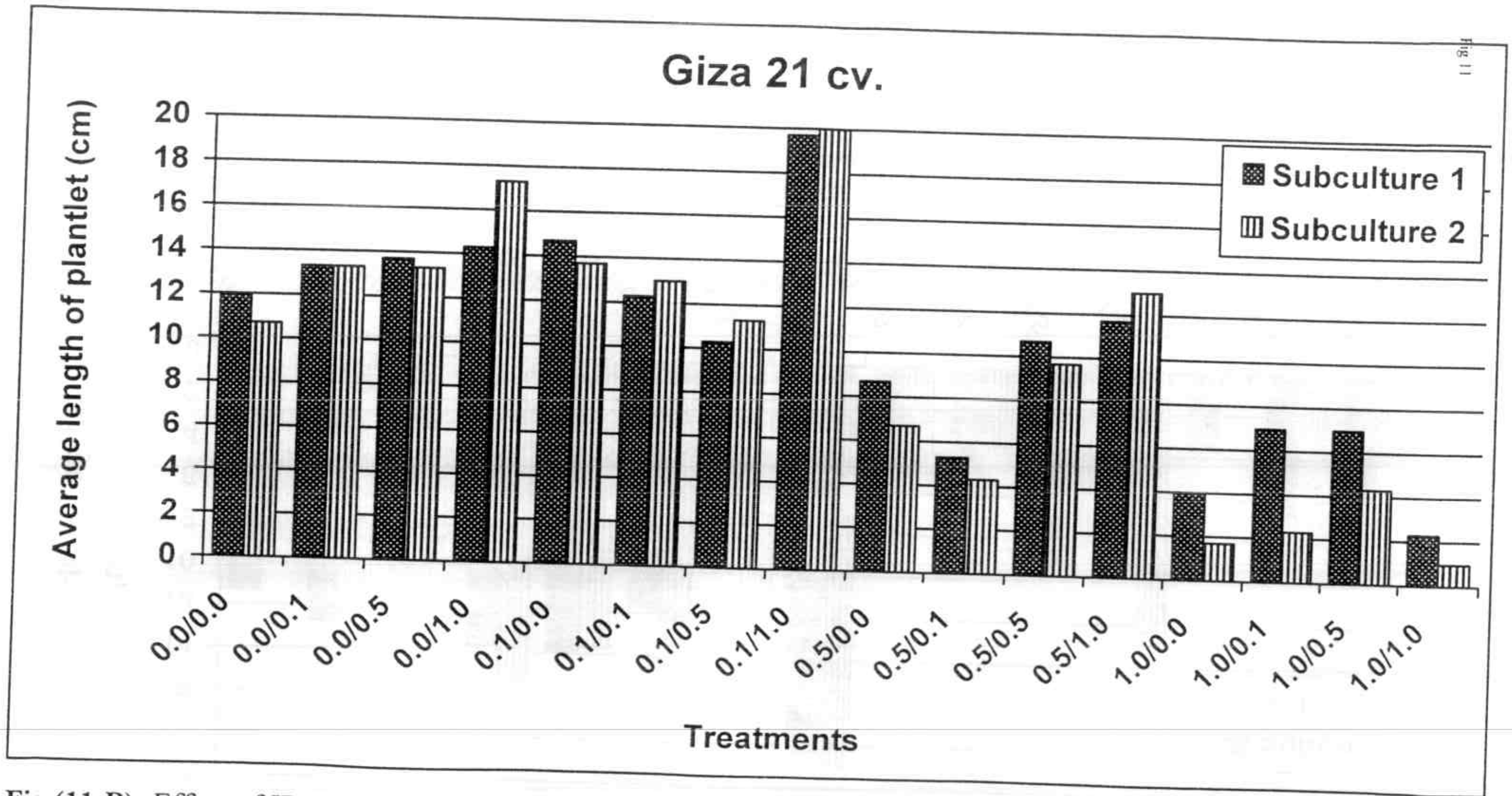


Fig (11-B): Effect of IBA and BA at different concentrations on the average length of adventitious shoots of watermelon Giza 21 cv. during two subcultures.

Regarding root formation the obtained results **(Table, 13)** and **Fig. (12)**, prove that the number of roots/plantlet (NRP) was sharply decreased when BA was combined (at any concentration) with IBA. However, in the absence of BA the NRP was increased as concentration of IBA was increased from 0.0 up to 1.0 mg/liter in comparison with the control treatment. This trend was more obvious in subculture1 more than subculture2 of both watermelon cultivars Giza 1 & Giza 21. The present results showed that using MS-medium contained 0.0 mg/liter BA + 1.0 mg/liter IBA resulted in the highest number of roots on plantlets of the two cultivars. Whereas, the lowest number was produced on a medium contained 1 mg BA combined with 0.0, 0.1, 0.5 or 1.0 mg/liter IBA.

Table (13): Effect of IBA and BA at different concentrations on the average number of roots/plantlet of 2 watermelon cvs. during two subcultures.

Treatments		Average number of roots/plantlet			
		Giza 1 cv.		Giza 21 cv.	
BA (mg/l)	IBA (mg/l)	Subculture 1	Subculture 2	Subculture 1	Subculture 2
0.0	0.0	4.3	2.7	2.3	2.7
0.0	0.1	4.7	5.0	4.3	5.3
0.0	0.5	5.3	6.3	7.3	7.0
0.0	1.0	7.3	10.7	8.7	12.7
0.1	0.0	2.3	1.7	1.3	2.3
0.1	0.1	3.3	4.0	3.7	5.3
0.1	0.5	4.7	4.7	3.7	6.7
0.1	1.0	5.3	6.7	5.7	7.7
0.5	0.0	1.3	1.0	1.3	2.3
0.5	0.1	1.3	0.7	2.0	0.3
0.5	0.5	2.3	3.0	1.7	4.3
0.5	1.0	2.3	2.3	1.3	1.3
1.0	0.0	0.3	0.0	0.0	0.3
1.0	0.1	0.7	0.3	0.3	0.7
1.0	0.5	0.0	1.0	0.0	1.3
1.0	1.0	1.7	0.7	0.3	0.3
LSD at 0.05 for		1.18	1.54	1.21	1.61

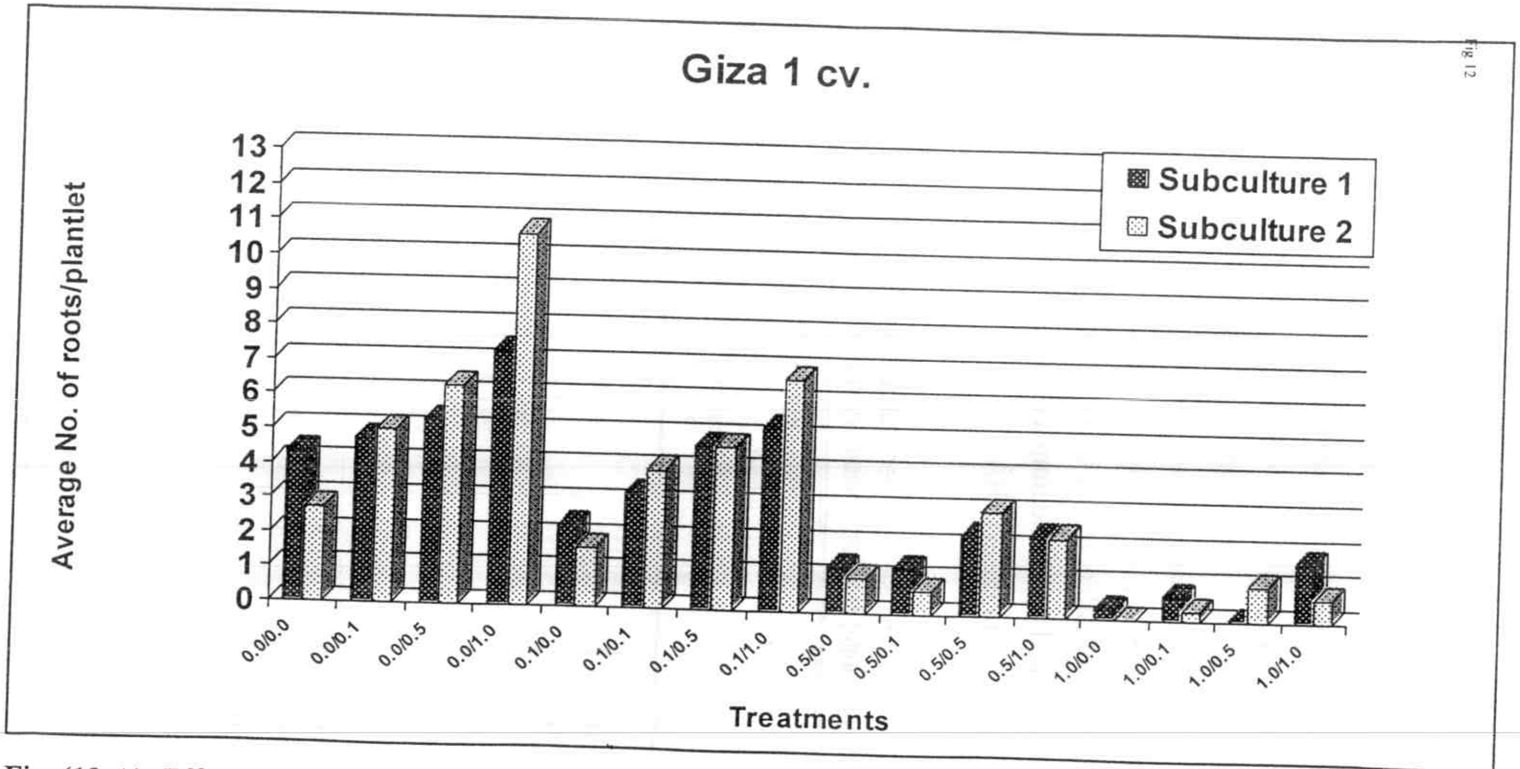


Fig. (12-A): Effect of IBA and BA at different concentrations on the average number of roots/plantlet of watermelon Giza 1 cv. during two subcultures.

Fig 12

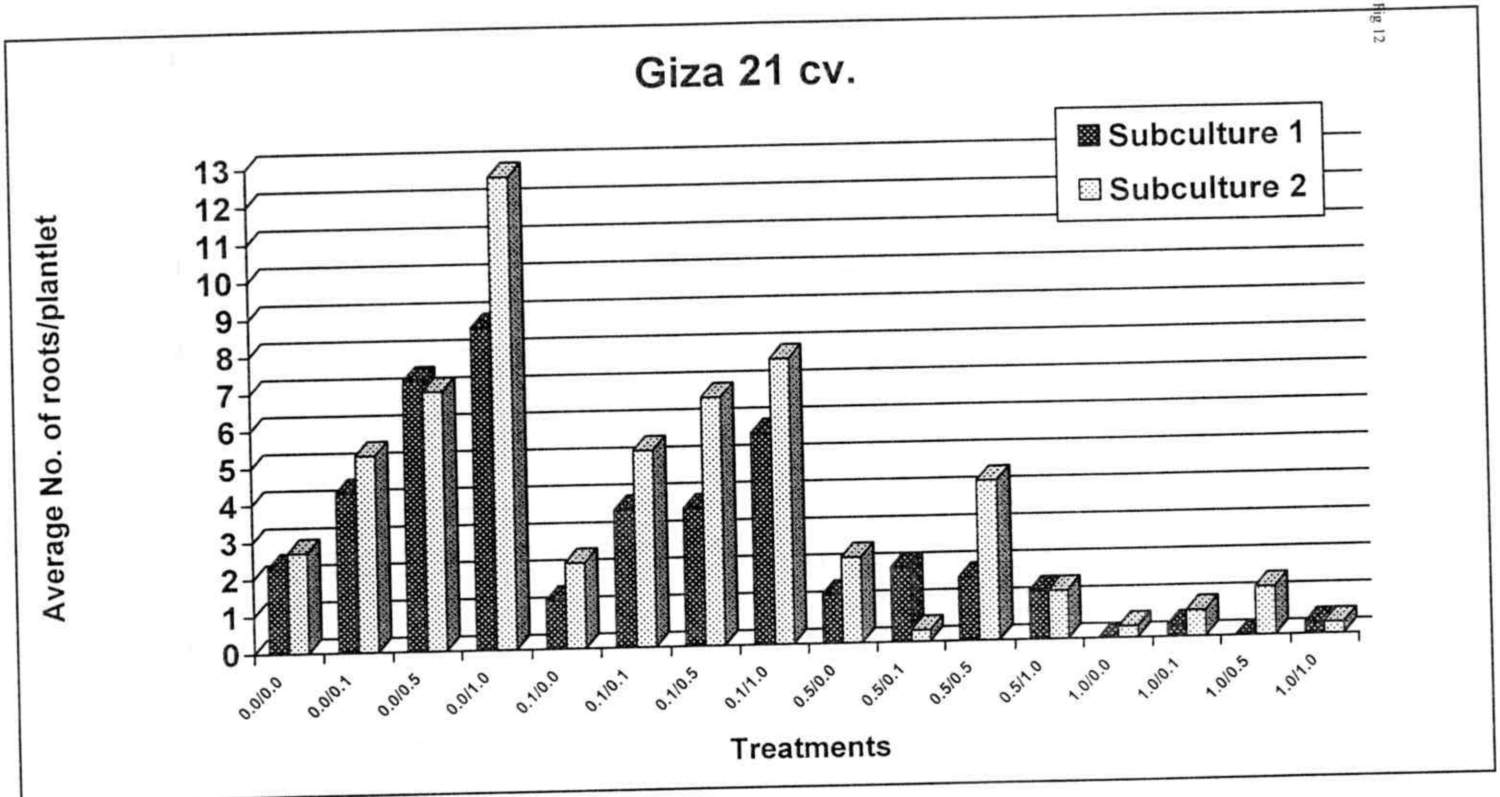


Fig. (12-B): Effect of IBA and BA at different concentrations on the average number of roots/plantlet of watermelon Giza 21 cv. during two subcultures.

The hyperhydricity values **(Table, 14)** and **Fig. (13)** were increased proportionally with increasing the concentration of BA and IBA either used each alone or in combination. This trend was more pronounced in subculture2 than subculture1 of both cultivars. In particular, the MS-medium contained BA at 1.0 mg/liter either alone or combined with IBA at 0.1, 0.5 or 1.0 mg/liter recorded the highest hyperhydricity value in both cultivars compared with the control treatment.

Table (14): Effect of IBA and BA at different concentrations on hyperhydricity values of 2 watermelon cvs. during two subcultures.

Treatments		Giza 1 cv.		Giza 21 cv.	
BA (mg/l)	IBA (mg/l)	Subculture 1	Subculture 2	Subculture 1	Subculture 2
0.0	0.0	1.0	1.0	1.0	1.0
0.0	0.1	1.0	1.0	1.0	1.0
0.0	0.5	1.0	1.3	1.0	2.0
0.0	1.0	1.0	2.0	1.0	1.7
0.1	0.0	1.0	1.7	1.7	3.7
0.1	0.1	1.0	1.3	1.0	2.0
0.1	0.5	1.0	2.0	1.7	2.7
0.1	1.0	1.0	2.0	2.0	3.0
0.5	0.0	1.7	3.3	2.7	5.0
0.5	0.1	1.3	2.3	2.0	4.0
0.5	0.5	1.7	3.0	2.3	4.7
0.5	1.0	1.7	4.0	3.7	5.0
1.0	0.0	2.7	5.0	3.0	5.0
1.0	0.1	2.0	4.3	2.7	5.0
1.0	0.5	3.0	5.0	3.3	5.0
1.0	1.0	3.0	5.0	3.7	5.0
LSD at 0.05 for		0.55	0.95	0.65	0.64

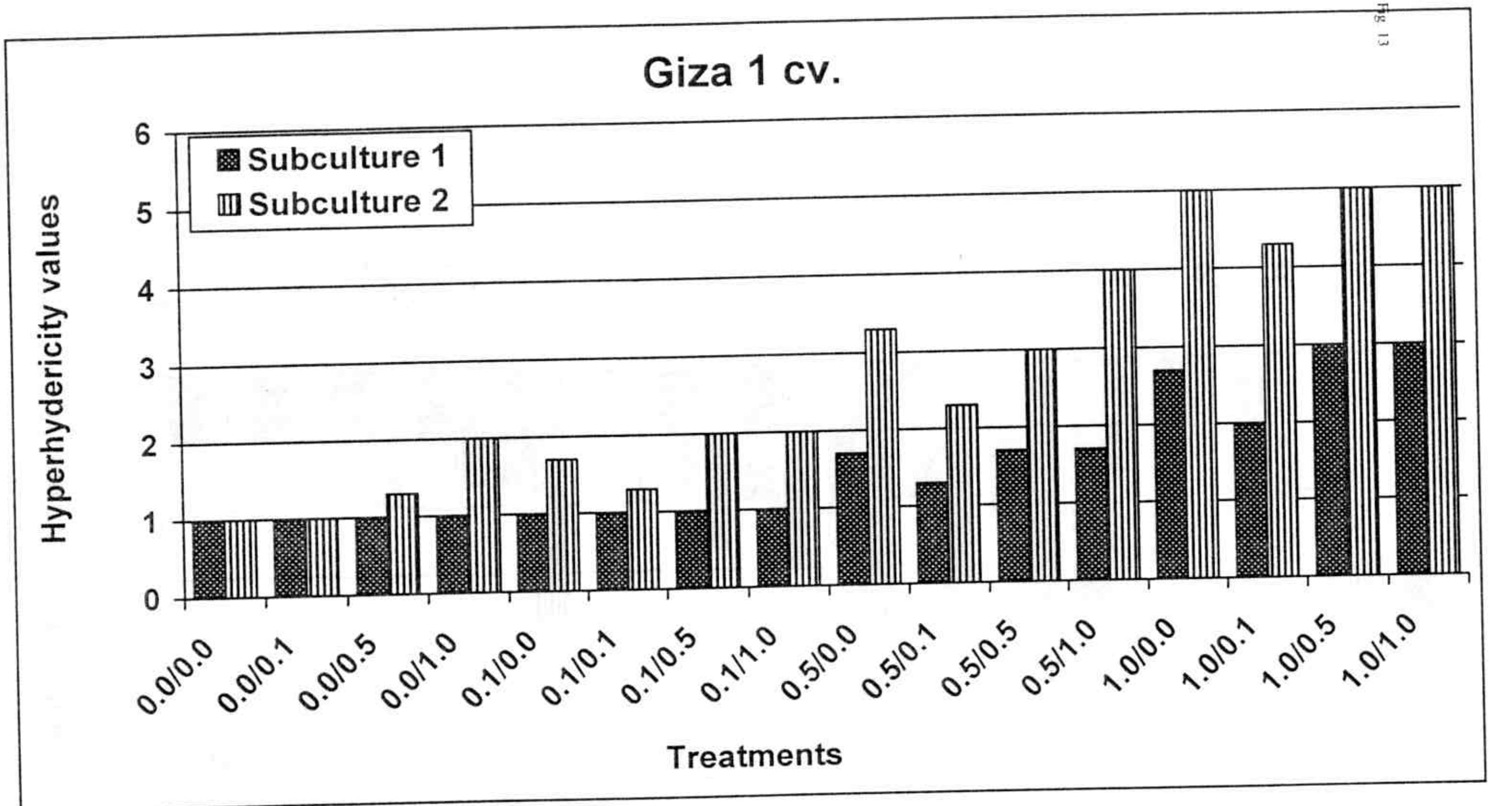


Fig. (13-A): Effect of IBA and BA at different concentrations on hyperhydricity values of watermelon Giza 1 cv. during two subcultures.

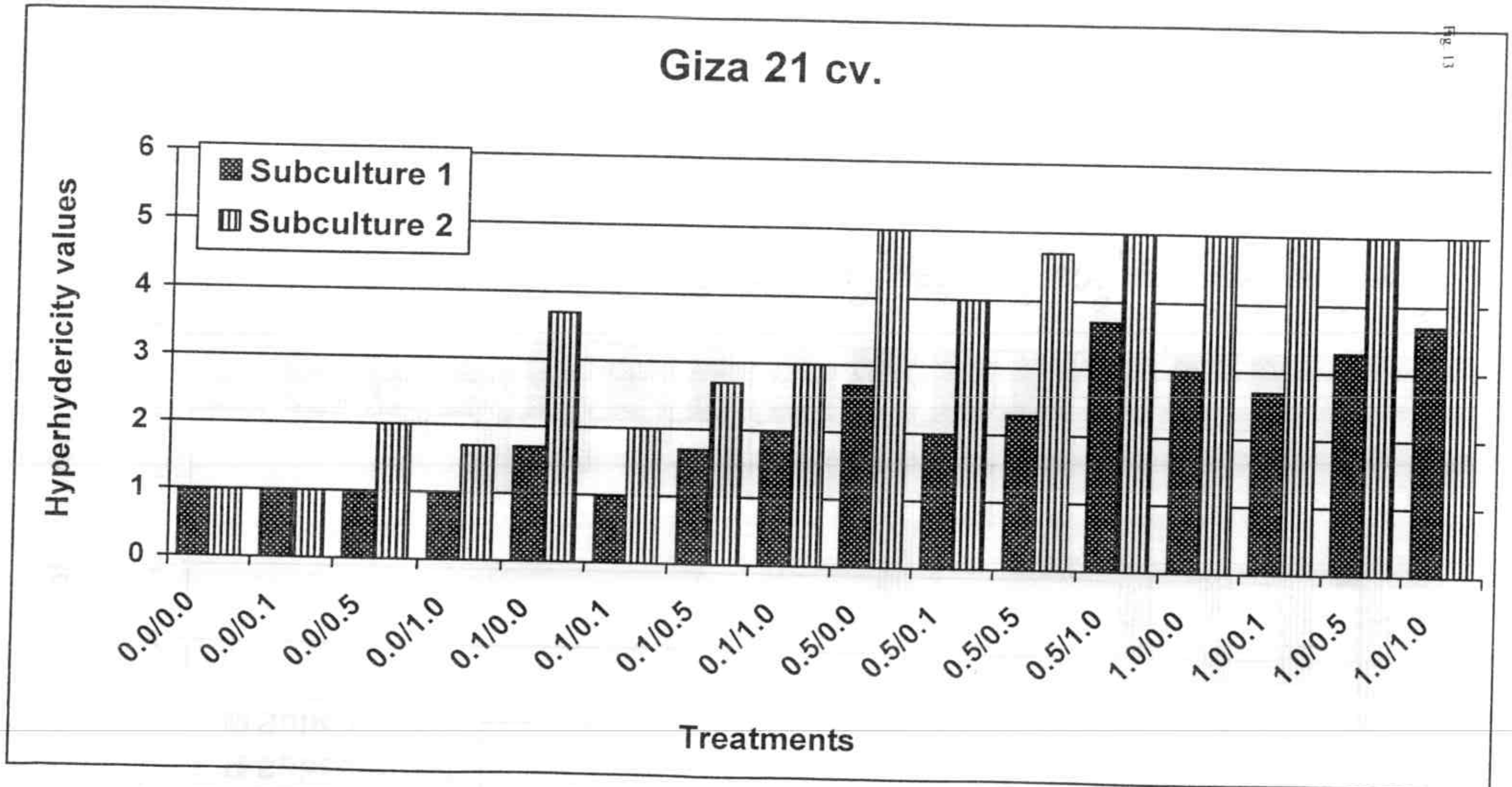


Fig. (13-B): Effect of IBA and BA at different concentrations on hyperhydricity values of watermelon Giza 21 cv. during two subcultures.

II.3. Effect of colchicine and dinitroaniline on production of the tetraploid watermelon plantlets:

In this experiment, explants (buds) were cultured for 9 days on liquid MS-medium containing 30g/l sucrose, 2 mg/liter BA and supplemented with different concentrations of Colchicine (10, 100 and 1000 ppm) or Dinitroaniline (5, 10 and 50 ppm) for regenerating watermelon tetraploid plants. Then the treated buds were cultured on solidified MS nutrient medium supplemented with 1.0 mg/liter IBA + 0.1 mg/liter BA. The shoots were subcultured every 21 – 30 days. The survival percentage, shoot number, shoot length, leaf number, callus formation and chloroplast number were determined. These parameters were recorded on plant production irrespective of their ploidy.

II.3.1. Growth characters of the regenerated tetraploid plantlets:

As for Giza1 cv., data in **Table (15)** show that the dinitroaniline and colchicine treatments significantly affected all the studied characters. Percentage of survival, shoot (number and length) and number of leaves/shoot and percentage of callus formation were not significantly affected by using the lowest concentrations of dinitroaniline (5ppm) and colchicine (10ppm) in comparison with the control treatment. However, using the highest concentration of colchicine (1000ppm) significantly decreased % survival (18.75), number (0.75) and length (1.45) of shoots and number of leaves/shoot (1.25) more than the concentration 100ppm which recorded 75.0%, 3.5, 3.13 and 11.25 concerning these characters, respectively. In contrast, % callus formation was significantly higher at 1000ppm (81.25%) than at 100ppm (37.5%). Data revealed that dinitroaniline at 50ppm significantly decreased survival (31.25%), number (3.75) and number of leaves/shoot (10.75) and increased % callus formation (37.5%) compared with the control treatment. The shoot length was not significantly affected by dinitroaniline concentrations compared with the control.

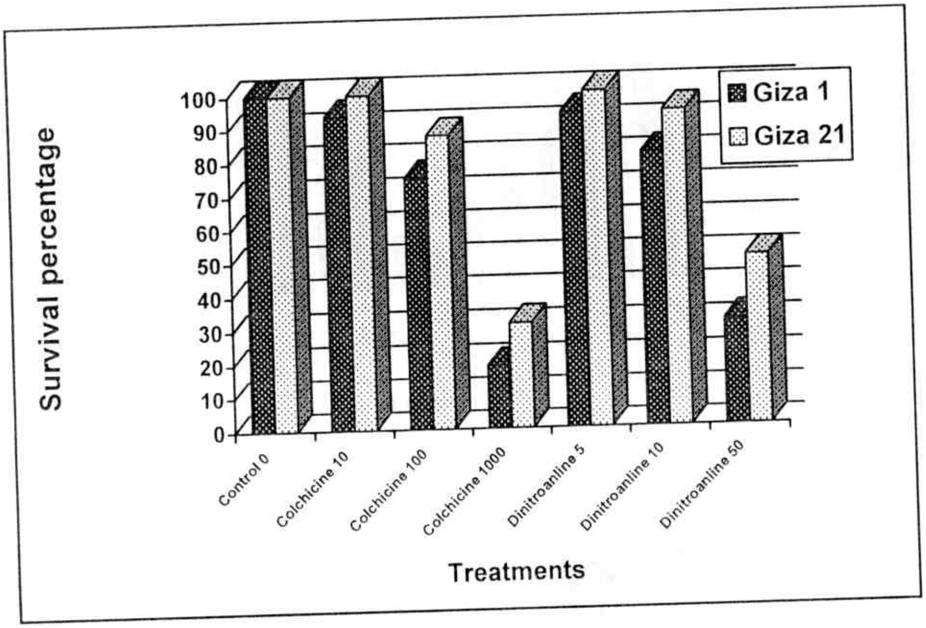
Data in **Table (16)** show that all studied characters of the watermelon Giza 21 cv., were affected with dinitroaniline and colchicine treatments in similar way as Giza1 cv. **(Table, 15)**. Using the highest concentrations of colchicine (1000ppm) and dinitroaniline (50ppm) caused the highest significant decrease in % survival (31.5 & 50.0%), shoot number (1.25 & 2.0) and shoot length (1.95 & 3.43) and number of leaves/shoot (3.25 & 7.0) compared with the control. On the contrary, % callus formation was significantly higher (68.7 & 37.5%) compared with the control (0.0%) **(Figs, 14 a and b)**.

Table (15): Effect of colchicine and dinitroaniline at different concentrations on vegetative growth parameters and % callus formation of watermelon cv. Giza 1.

Treatments (growth regulators and concentrations in ppm)		Survival (%)	Shoot number	Shoot length (cm)	Leaves number	Callus formation (%)
Control	0	100.00	6.50	5.80	19.75	0.00
Colchicine	10	93.75	6.50	5.50	18.00	18.75
	100	75.00	3.50	3.13	11.25	37.50
	1000	18.75	0.75	1.45	1.25	81.25
Dinitroaniline	5	93.75	6.75	4.20	21.50	6.25
	10	81.25	7.00	4.73	19.50	12.50
	50	31.25	3.75	4.20	10.75	37.50
LSD at 0.05		17.97	2.22	1.98	4.78	19.29

Table (16): Effect of colchicine and dinitroaniline at different concentrations on vegetative growth parameters and % callus formation of watermelon cv. Giza 21.

Treatments (growth regulators and concentrations in ppm)		Survival (%)	Shoot number	Shoot length (cm)	Leaves number	Callus formation (%)
Control	0	100.00	4.75	6.60	19.00	0.00
Colchicine	10	100.00	5.25	5.58	17.50	0.00
	100	87.50	5.00	2.88	10.00	31.25
	1000	31.25	1.25	1.95	3.25	68.75
Dinitroaniline	5	100.00	5.25	5.53	22.75	6.25
	10	93.75	7.75	4.30	17.50	18.75
	50	50.00	2.00	3.43	7.00	37.50
LSD at 0.05		11.69	1.58	0.58	4.36	17.19



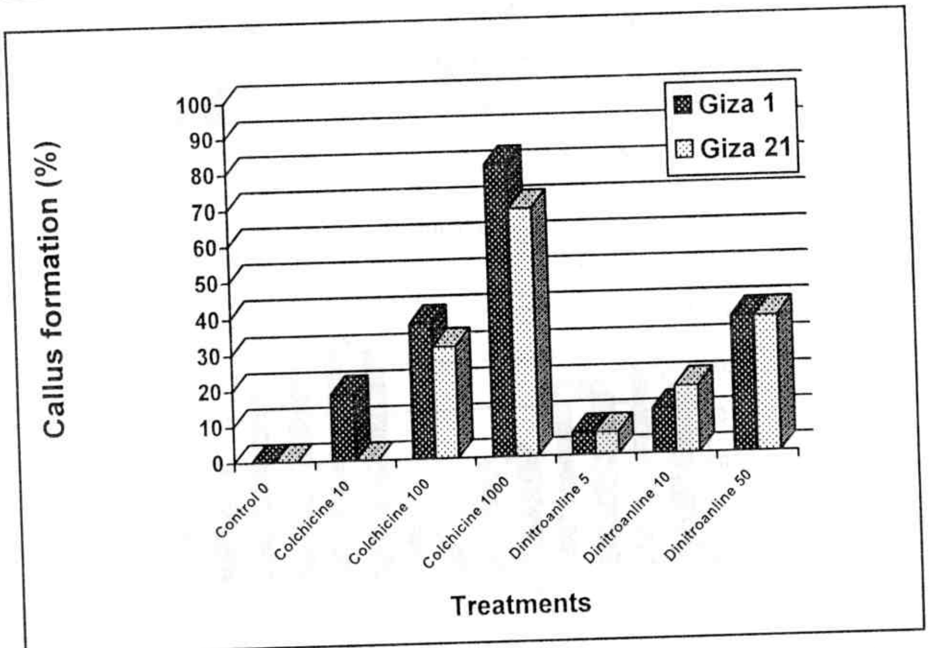


Fig (14a): Effect of colchicine and dinitroaniline at different concentrations on percentage of survival and callus formation of watermelon cvs. Giza 1 and Giza 21.

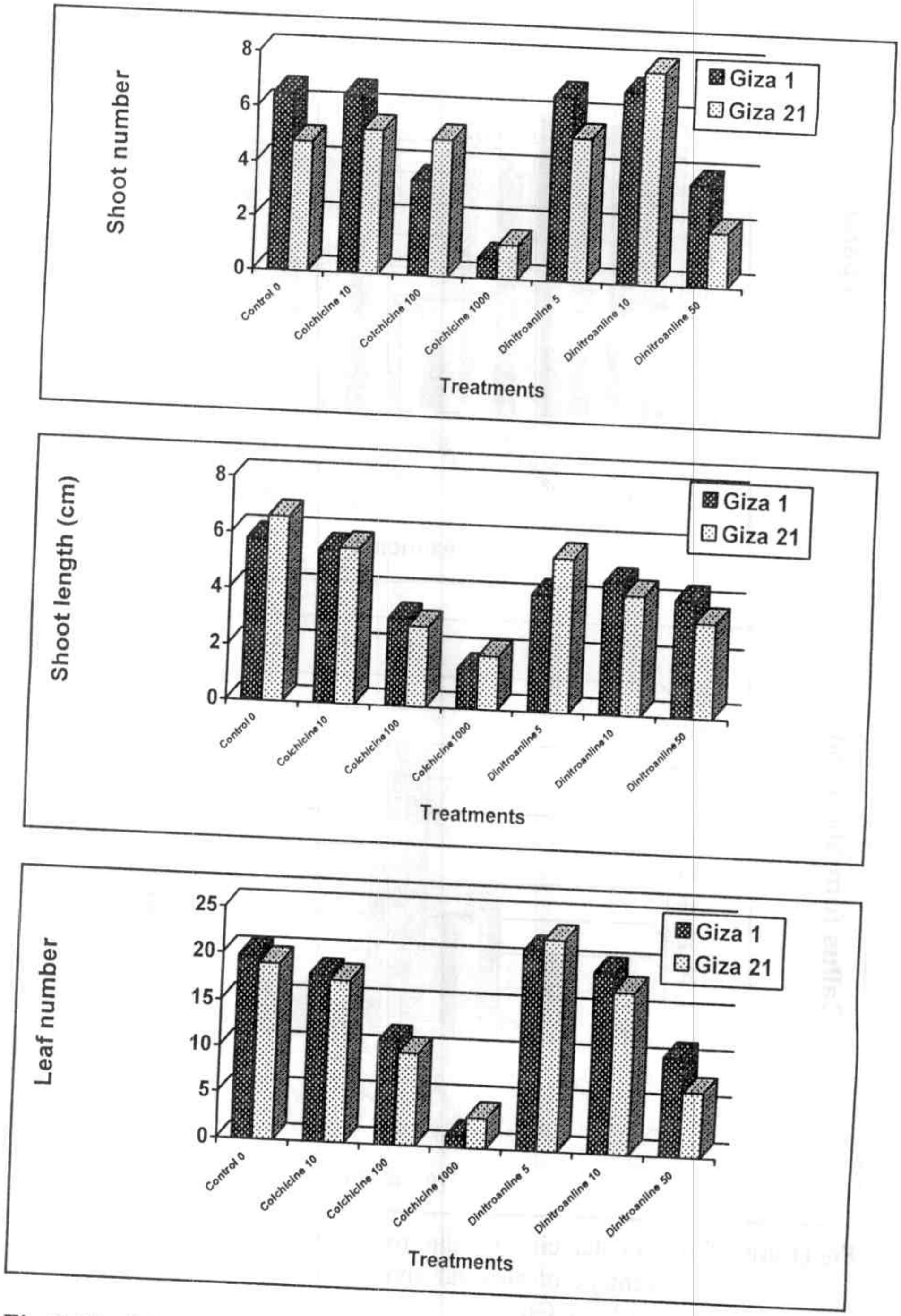


Fig (14b): Effect of colchicine and dinitroaniline at different concentrations on vegetative growth parameters of watermelon cvs. Giza 1 and Giza 21.

II.3.2. Examination for inspecting tetraploid plants:

Data in **Table (17)** show that the MS-medium without dinitroaniline or colchicine did not generate any tetraploid plantlets whereas, all plantlets were of diploid type. However, the tetraploid plantlet type was generated only on the MS-medium treated with colchicine or dinitroaniline. Percentages of the tetraploid plantlets were increased proportionally with increasing concentrations of colchicine and dinitroaniline added to the culture, MS-medium. Number of chloroplasts in the stomatal guard cells was obviously higher in tetraploid than diploid plantlets. Thus, adding colchicine to the culture MS-medium at concentrations of 10, 100 to 1000 ppm led to generate tetraploid plantlets at rates of 40, 60 to 70% in case of Giza 1 cultuvar and 30.0, 40.0 and 60.0% in case of Giza 21 cultivar, respectively. Number of chloroplast was ranged 18.8-19.5 and 19.0-19.9 in the tetrtraploid plantlets of Giza 1 and Giza 21 cvs., compared with 10.8-13.4 and 9.2-11.8 in the diploid plantlet type of both cultivars, respectively **(Photo, 3)**.

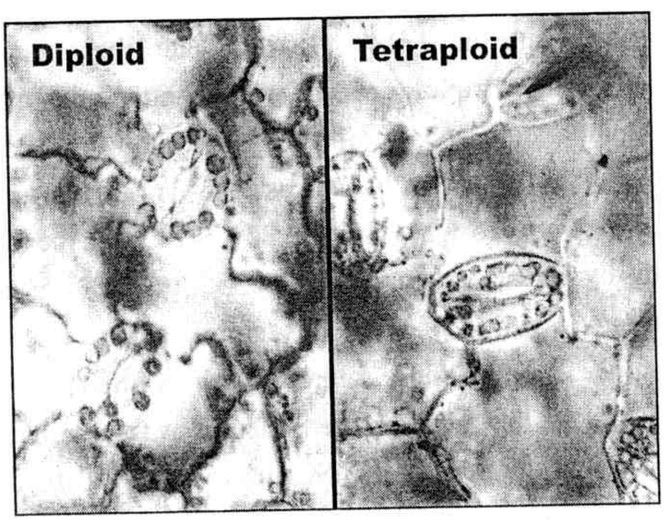


Photo (3): Chloroplast number in guard cells of diploid and tetraploid watermelon plants.

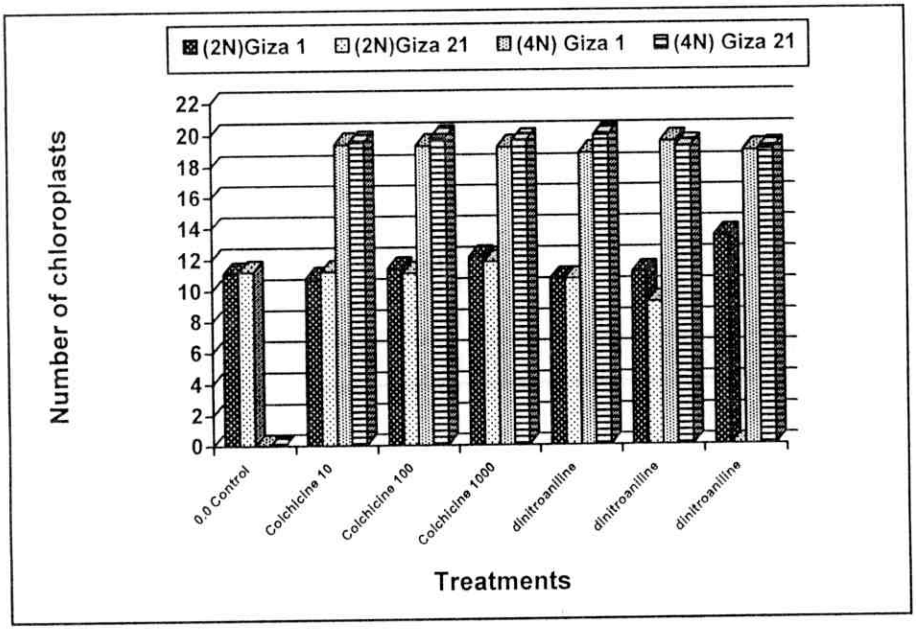
Table (17): Effect of colchicine and dinitroaniline at different concentrations on percentage of diploid and tetraploid plantlet formation in 2 local watermelon cultivars.

Treatments (conc. ppm)		Number of chloroplasts				% Plantlet genotype			
		Diploid (2N)		Tetraploid (4N)		Diploid (2N)		Tetraploid (4N)	
		Giza 1	Giza 21	Giza 1	Giza 21	Giza 1	Giza 21	Giza 1	Giza 21
Control	0.0	11.1	11.2	0.0	0.0	100	100	0.0	0.0
Colchicine	10	10.8	11.2	19.4	19.5	60	70	40	30
	100	11.4	11.1	19.3	19.8	40	60	60	40
	1000	12.1	11.8	19.2	19.6	30	40	70	60
dinitroaniline	5	10.7	10.7	18.8	19.9	70	80	30	20
	10	11.1	9.2	19.5	19.2	50	50	50	50
	50	13.4	0.0	18.9	19.0	10	0	90	100

II.4. Production of triploid (seedless) watermelon adventitious shoots via direct organogenesis:

II.4.1. Germination of triploid seeds:

In this study, surface sterilized seeds of triploid watermelon were placed into small jars (10 seeds/jar) containing autoclaved cotton pieces saturated with distilled water or liquid MS basal salts and incubated at different temperatures. Percentage of seed germination was recorded. Data in **Table (18)** indicate that percentage of germination of the triploid seeds was significantly affected by incubation temperature and germination-substrate as well as by their interaction. It was significantly increased as incubation temperature was increased from 25°C (6.0%) to 32°C (59.0%) then decreased significantly by elevating temperature to 35°C (34.0%). Using cotton pieces saturated with MS salts was significantly better for seed germination (29.2%) than those saturated with distilled water (16.0%). In general, the highest % seed germination was obtained at 32°C either on cotton saturated with MS salts (62.0%) or distilled water (56.0%) without significant differences in between.



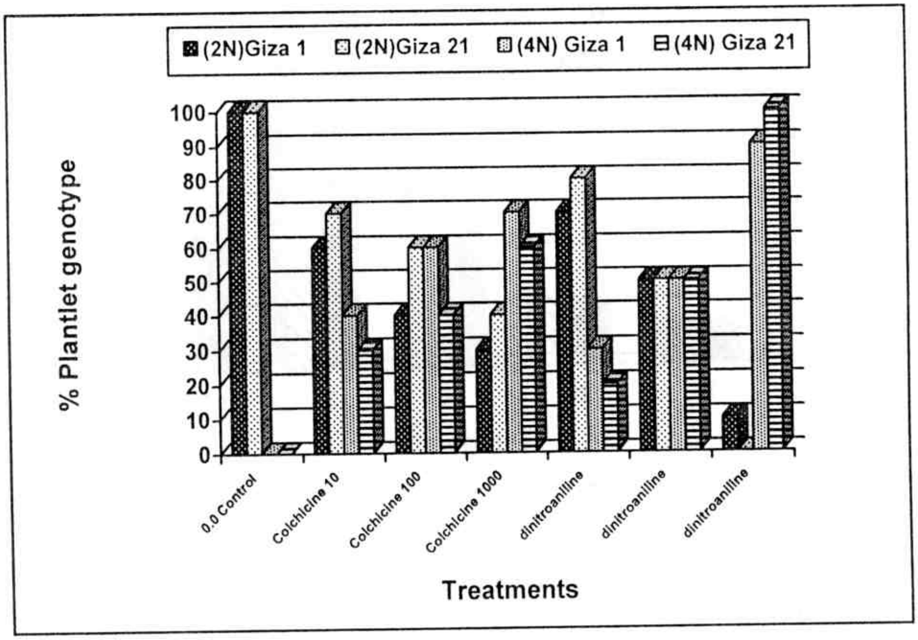


Fig (15): Effect of colchicine and dinitroaniline (at different concentrations) on number of chloroplasts and percentage of diploid and tetraploid plantlet formation in 2 local watermelon cultivars.

Table (18): Effect of medium type and temperature on germination percentage of triploid seeds.

Temperature (°C) (A)	Medium type		Mean
	Water	MS salts	
25	6.0	6.0	6.0
28	10.0	12.0	11.0
30	26.0	34.0	30.0
32	56.0	62.0	59.0
35	32.0	36.0	34.0
Mean (B)	26.0	29.2	

L.S.D. at 0.05:

Temperature (T) 3.69
Media (M) 2.83
Interaction TxM 8.25

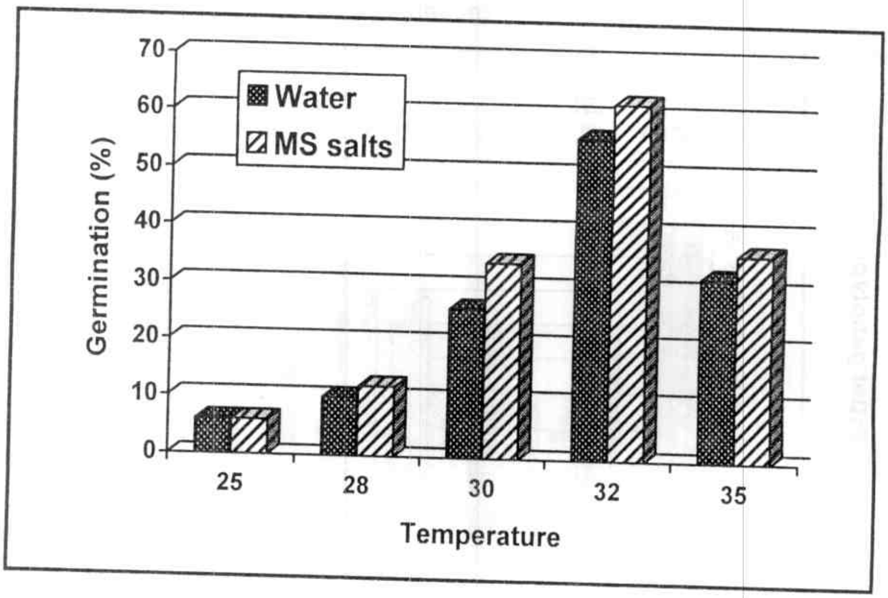


Fig (16): Effect of media type and temperature on germination percentage of triploid seeds.

II.4.2. Effect of cytokinin types and concentrations on the production of the adventitious shoots of seedless (triploid) watermelon:

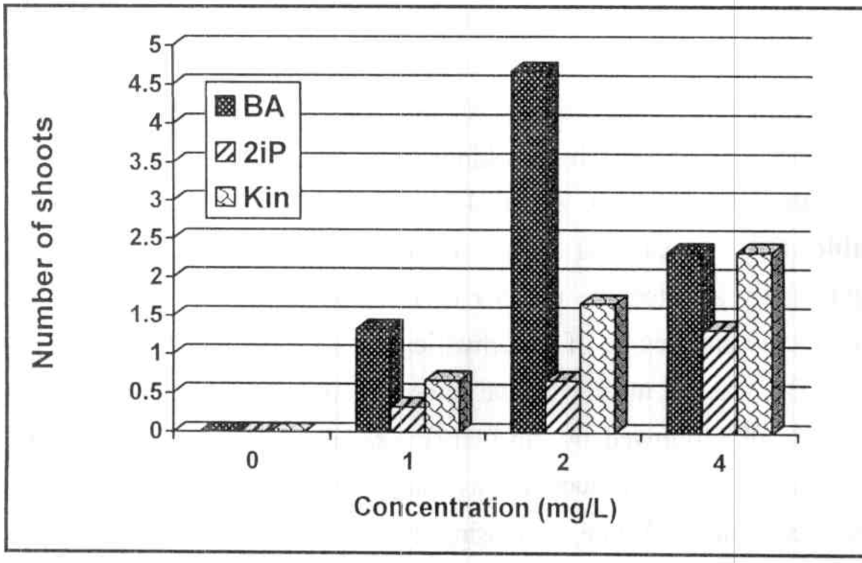
Number of adventitious shoots and hyperhydricity of triploid watermelon as affected by cytokinin types (BA, 2iP or kin) at different concentrations (0.0, 1.0, 2.0 or 4.0 mg/liter) was investigated. Data in **Table (19)** reveal that tested cytokinins and their concentrations significantly affected the number of the adventitious shoots of triploid watermelon and degree of hyperhydricity. As for number of adventitious shoot, BA was the most effective since it recorded the highest number of shoots (2.08) followed by kin (1.17) and 2iP (0.58), respectively. It is clear that the shoot production was completely inhibited on the cytokinin free MS-medium. While, increasing cytokinin concentration to 1.0, 2.0 and 4.0 mg/liter significantly increased number of the developed shoots to 0.78, 2.34 and 1.99, respectively without significant differences between the latter two concentrations. In general, BA at 2.0 mg/liter produced the highest number of shoots (4.67) followed by BA and kin at 4.0 mg/liter (2.33).

Data indicate also that the incidence of hyperhydricity was significantly increased by increasing concentrations of any cytokinins tested compared with the control treatment (without cytokinins). Using BA particularly at the higher concentration (4.0 mg/liter) caused the highest degree of hyperhydricity (4.0).

Table (19): Effect of cytokinin types and concentrations on adventitious shoot formation and hyperhydericity of triploid (seedless) watermelon.

Conc. (mg/l)	Shoot number				Hyperhydricity value			
	BA	2iP	kin	Mean	BA	2iP	kin	Mean
0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0
1.0	1.33	0.33	0.67	0.78	2.33	1.67	1.67	1.89
2.0	4.67	0.67	1.67	2.34	2.33	2.33	2.33	2.33
4.0	2.33	1.33	2.33	1.99	4.00	2.67	2.33	3.00
Mean	2.08	0.58	1.17		2.42	1.92	1.83	

L.S.D. at 0.05:	Shoot N	Hyperhydericity
Cytokinin (N)	0.72	0.43
Concentration (C)	0.62	0.37
Interaction NxC	1.52	0.75



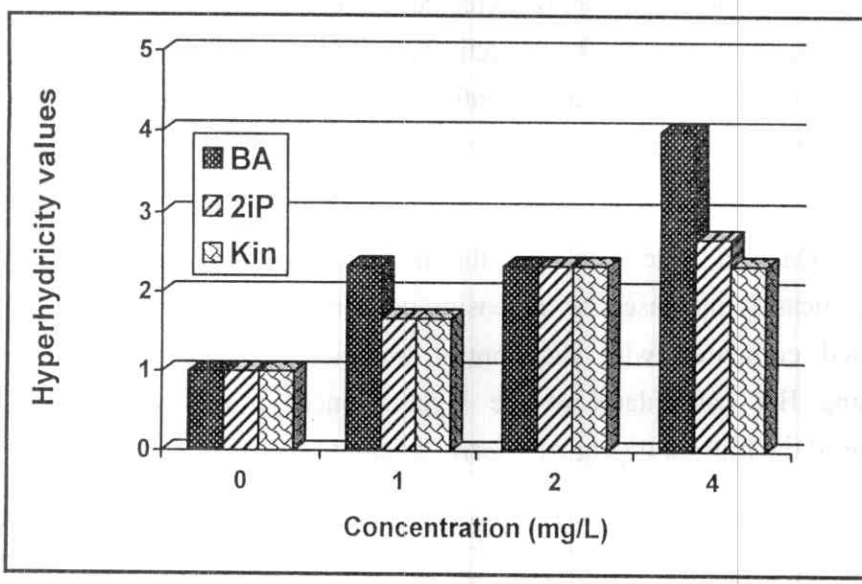


Fig (17): Effect of cytokinin types and concentrations on adventitious shoot formation and hyperhydericity of triploid (seedless) watermelon.

II.5. Production of diploid adventitious shoot via indirect organogenesis:

II.5.1. Effect of different concentrations and combinations of BA and IBA on percentage of callus formation:

Data in **Table (20)** clearly show that callus formation was greatly varied and depended on the used plant materials and tested growth regulators as well as their combination. Using hypocotyles as explants formed higher percentage of callus formation than cotyledon explants. Average percentages of callus formation from hypocotyle explants of Giza 1 and Giza 21 were 35.01 and 37.08% compared with cotyledon explants of both cultivars *i.e.* 26.24 and 26.68%, respectively.

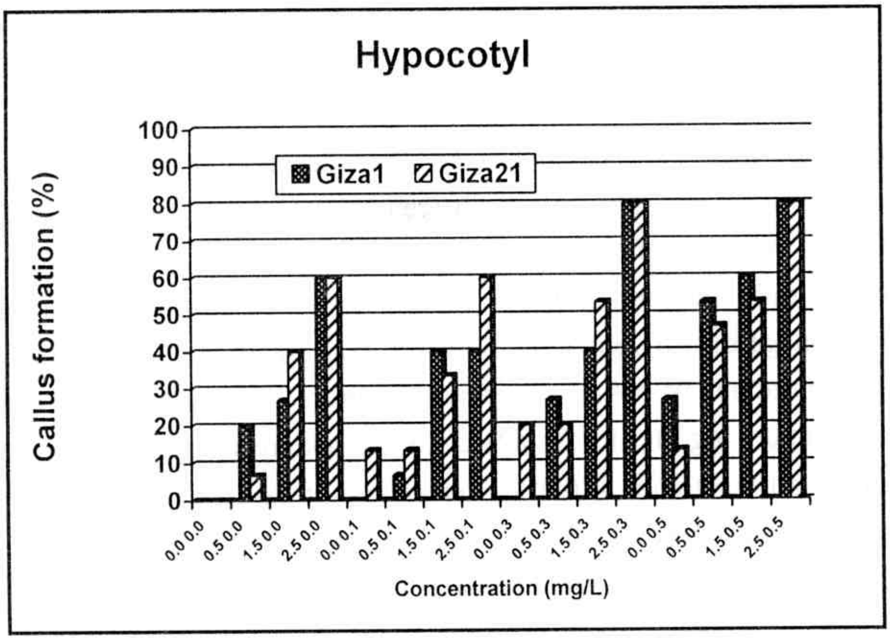
Averages of callus formation were increased proportionally with increasing the tested concentrations of BA. In MS-medium free from BA, averages of callus formation from hypocotyle and cotyledon explants of Giza 1 cv., were 6.68 and 1.68%, respectively. As for Giza 21 cv., the corresponding figures on the same BA free medium were 11.65 and 6.68% for the two kinds of explants, respectively. Averages of callus formation were increased to 26.68, 41.68 and 65.0% (in Giza 1 cv.) and 21.68, 44.98 and 70.0% (in Giza 21 cv) when hypocotyle explants of both cultivars were cultured on MS-medium contained BA at concentrations of 0.5, 1.5 and 2.5 mg/liter, respectively. While, cotyledon explants yielded 20.0, 29.98 and 53.33% callus formation in Giza 1 cv., and 18.33, 35.03 and 46.68% in Giza 21 cv., when they were cultured on MS-medium supplemented with these three concentrations of BA, respectively.

IBA at different concentrations (0.1, 0.3 and 0.5 mg/liter) seemed to be, in general, less effective on callus formation of both cultivars when compared with control (free IBA-medium). The averages of callus formation were 23.35-26.68% in Giza 1 cv., and 29.98-30.0% in Giza 21 cv., when hypocotyle explants were cultured on MS-medium treated with 0.1, 0.3 and 0.5 mg/liter of IBA, respectively. However, the averages were ranged 18.35-23.33% in the first cultivar and 15.0-20.0% in the second one when their cotyledon explants were cultured on medium treated with the three concentrations, respectively. It is interest to state that the averages of callus formation were 26.68 and 18.35% in Giza 1 cv and 26.68 and 15.0% when hypocotyle and cotyledon explants, respectively were cultured on IBA free medium.

Results of the present investigation show that the callus formation was completely failed on MS-medium free from both BA and IBA. However, the callus formation was slightly increased by increasing concentrations of IBA and further increases were obtained by increasing concentration of BA. Thus, MS- medium supplemented with the highest concentration of BA and IBA (2.5 mg/liter BA + 0.5 mg/liter IBA) and cultured with hypocotyle of both Giza 1 and Giza 21 cvs., produced the highest percentages of callus formation (80.0%), meanwhile cotyledon explants produced 73.3 and 66.7% callus formation for both cultivars, respectively **(Photo, 4)**.

Table (20): Effect of different concentrations and combinations of BA and IBA on percentage of callus formation from hypocotyles and cotyledons of watermelon cultivars Giza1 and Giza21.

Treatments (mg/l)		Plant materials				Mean	
		Hypocotyles		Cotyledons			
BA	IBA	Giza1	Giza21	Giza1	Giza21	Hypocotyles	Cotyledons
0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.00
0.5	0.0	20.0	6.7	6.7	0.0	13.35	3.35
1.5	0.0	26.7	40.0	20.0	26.7	23.35	33.35
2.5	0.0	60.0	60.0	46.7	33.3	53.35	46.65
0.0	0.1	0.0	13.3	0.0	0.0	0.00	6.65
0.5	0.1	6.7	13.3	13.3	20.0	10.00	16.65
1.5	0.1	40.0	33.3	33.3	26.7	36.65	30.00
2.5	0.1	40.0	60.0	40.0	40.0	40.00	50.00
0.0	0.3	0.0	20.0	0.0	6.7	0.00	13.35
0.5	0.3	26.7	20.0	26.7	13.3	26.70	16.65
1.5	0.3	40.0	53.3	33.3	40.0	36.65	46.65
2.5	0.3	80.0	80.0	53.3	46.7	66.65	63.35
0.0	0.5	26.7	13.3	6.7	20.0	16.70	16.65
0.5	0.5	53.3	46.7	33.3	40.0	43.30	43.35
1.5	0.5	60.0	53.3	33.3	46.7	46.65	50.00
2.5	0.5	80.0	80.0	73.3	66.7	76.65	73.35
	L S D at 0.05	13.89	14.00	15.47	13.32		



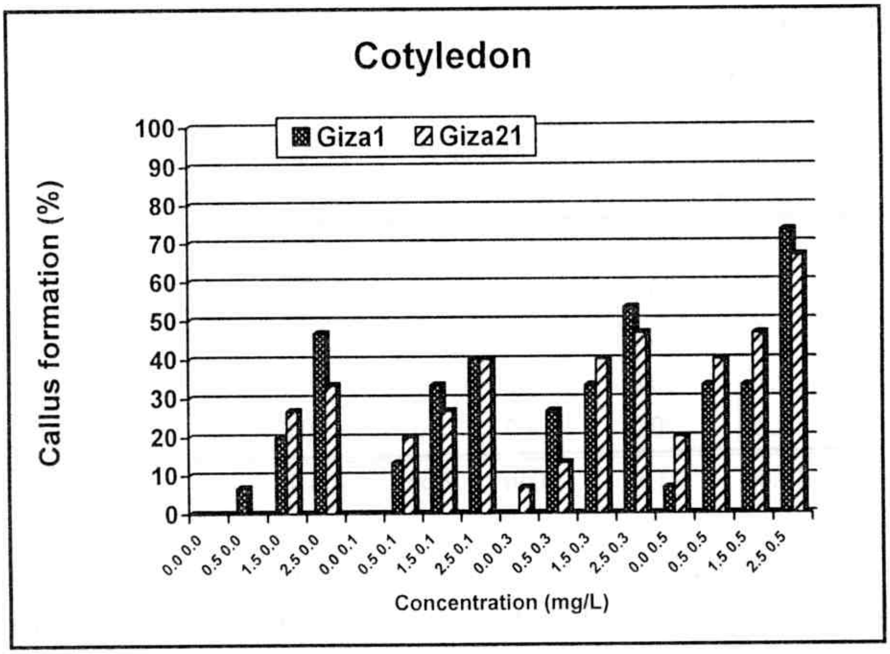


Fig (18): Effect of different concentrations and combinations of BA and IBA on percentage of callus formation from hypocotyles and cotyledons of watermelon cultivars Giza1 and Giza21.

II.5.2. Effect of naphthalene acetic acid (NAA) on callus differentiation.

Data in **Table (21)** show that percentage of callus differentiation, number of shoots and roots in both watermelon cultivars were significantly affected by concentrations of NAA in the culture medium. The callus differentiation in watermelon cultivar Giza21 was completely inhibited while, Giza1 cv., showed slight callus differentiation (20.0%) on the NAA-free medium (Control). Percentage of differentiation, however, was significantly increased as concentrations of NAA was increased up to 0.05 mg/liter for Giza1 cv., and 0.1 mg/liter for Giza21 cv., since both recorded the highest % callus differentiation (80.0%). Percentage of callus differentiation in both cultivars was significantly decreased to 20.0% by elevating NAA concentration in the culture media up to 0.2 mg/liter.

Table (21): Effect of different concentrations of NAA on percentage of callus differentiation, number of shoots and roots.

NAA (Conc. mg/l)	Giza 1			Giza 21		
	% Differentiation	Number of		% Differentiation	Number of	
		Shoots	Roots		Shoots	Roots
0.00	20.0	0.8	0.0	0.0	0.0	0.0
0.01	60.0	2.4	3.6	40.0	1.2	3.2
0.05	80.0	3.6	1.2	60.0	5.0	2.2
0.10	60.0	1.8	3.6	80.0	3.8	5.2
0.20	20.0	1.2	5.0	20.0	1.4	6.4
L S D at 0.05	13.56	0.97	2.38	14.17	1.37	2.78

Similarly, the number of shoots and callus was affected by NAA. Using NAA at 0.05 mg/liter produced the highest number of shoots/callus in Giza1 cv. (3.6) and Giza21 (5.0). Increasing NAA concentration to 0.02 mg/liter significantly decreased number of shoots/callus to 1.2 (Giza1) and 1.4 (Giza21). The number of shoots/callus was obviously higher in Giza21 than Giza1 particularly at the concentrations (0.05 & 0.1 mg/liter) of NAA.

As for root formation, the results proved that it was completely suppressed in the absence of NAA whereas it was maximized in both watermelon cultivars Giza1 (5.0) and Giza21 (6.4) by elevating NAA concentration up to 0.2 mg/liter **(Photo, 4).**

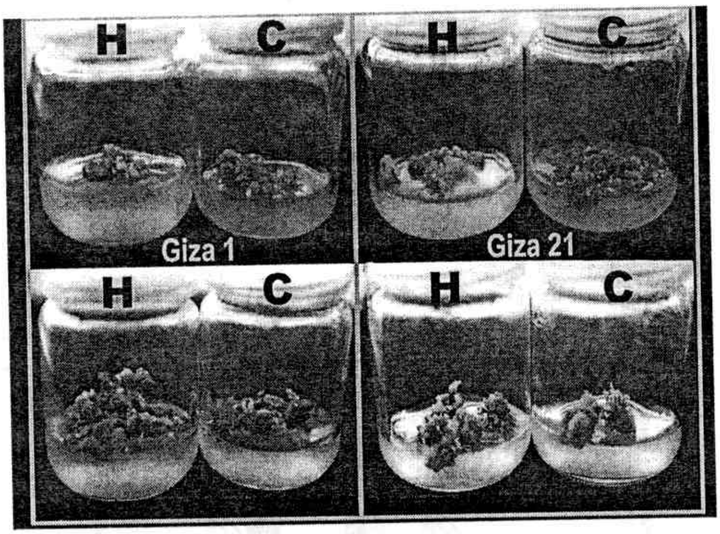


Photo (4): Callus formation and adventitious shoot regeneration in watermelon Giza 1 and Giza 21 cvs. from hypocoytl (H) and cotyledon (C).

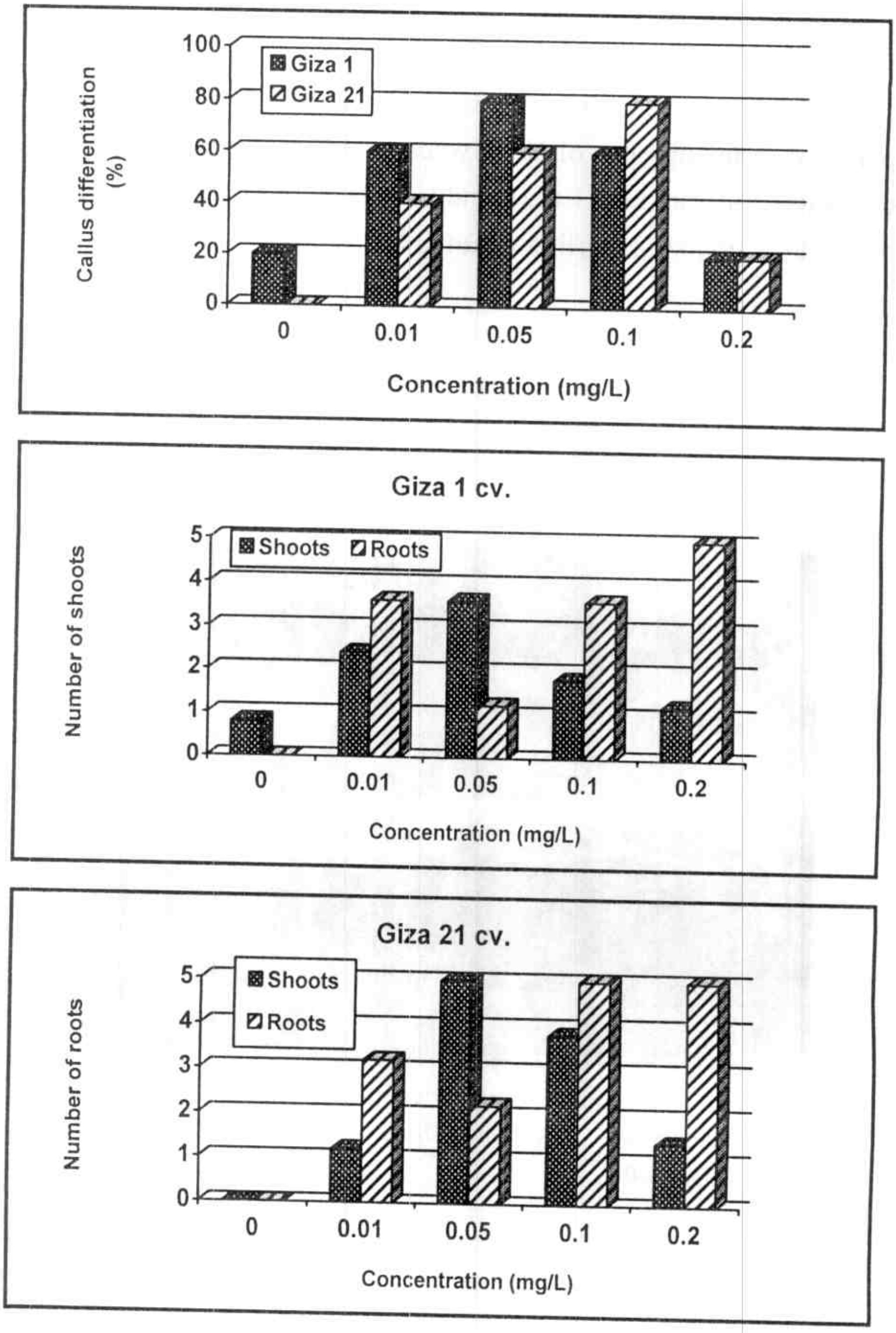


Fig. (19): Effect of different concentrations of NAA on percentage of callus differentiation, number of shoots and roots.

Part III. Screening for resistance against watermelon fusarium wilt *in vitro* and *ex vitro*:

III.1. Effect of different concentrations of cultural filtrate of the wilt pathogen on watermelon shoots:

In this study, different concentrations of *F. oxysporum nevium* culture filtrate were added aseptically to the MS-medium to estimate their effect on watermelon shoots of Giza 1 and Giza 21 cultivars. Data in **Table (22)** reveal that percentages of dead (wilted) shoots were significantly increased as concentration of cultural filtrate was increased. More than 99.0% of watermelon shoots were dead by adding cultural filtrate to the culture medium at the rate of 40 ml/l compared with the control treatment (without filtrates). The percentage of dead shoots was significantly higher in Giza21 cv. (55.07%) than Giza1 cv. (53.46%). The incidence of shoot death was not significantly affected by the interaction between watermelon cultivars and concentrations of culture filtrates.

Table (22): Effect of different concentrations of *F. oxysporum nevium* culture filtrate on percentage of wilted (dead) shoots of watermelon Giza 1 and Giza 21 cvs.

Conc. of culture filtrates (ml/l)	% Dead shoots		Mean
	Giza 1 cv.	Giza 21 cv.	
0	0.00	0.00	0.00
5	1.3	2.0	1.65
10	6.0	8.7	7.35
15	20.0	20.3	20.15
20	28.7	34.0	31.35
25	38.0	41.7	39.85
30	52.3	51.3	51.8
35	89.0	92.7	90.85
40	99.3	100.0	99.65
45	100.0	100.0	100.00
50	100.0	100.0	100.00
Mean	53.46	55.07	

L.S.D. at 0.05 for:

Concentration of CF (A) 1.99
Cultivars (B) 0.89
A x B n.s.

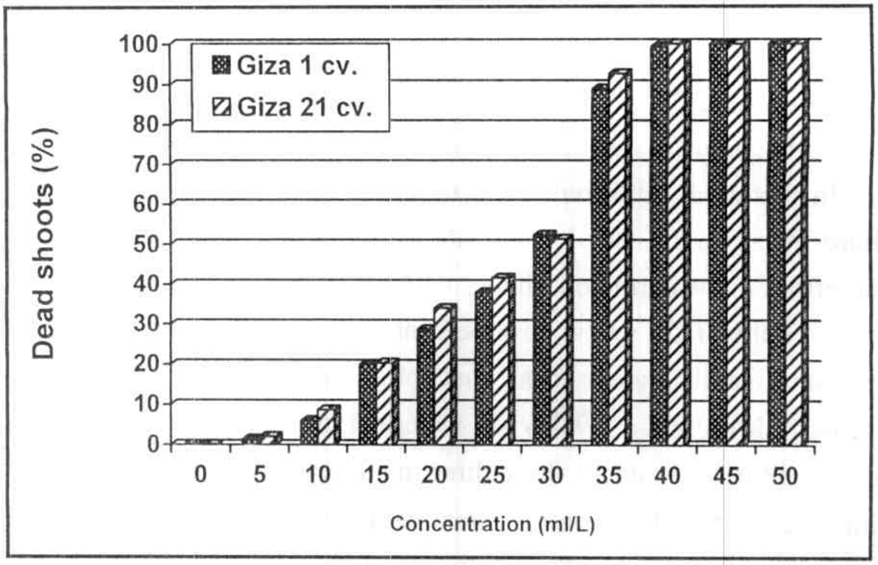


Fig. (20): Effect of different concentrations of *F. oxysporum nevium* culture filtrate on percentage of wilted (dead) shoots of watermelon Giza 1 and Giza 21 cvs.

III.2. Evaluating watermelon shoots regenerated from different explant sources for Fusarium wilt resistance:

In this experiment, Giza 1 and Giza 21 shoots (500) of diploid type [from adventitious shoots of cotyledons and callus) and 100 shoots of triploid type (Giza 1 cv.) were cultured on MS-nutrient medium containing *F. oxysporum nevium* culture filtrate at the rate of 40 ml/l. Percentage of shoots that still survived after 2 weeks on the treated media (resistant) were determined. Data in **Table (23)** reveal that shoots of triploid type (Giza 1 cv.) were the most resistant as they exhibited the highest percentage of survival (91.0%) while, shoots of tetraploid type (both Giza 1 and Giza 21 cvs.) were completely susceptible (100.0% death). On the contrary, appreciable resistance against the used culture filtrates were detected between the diploid shoots developed from adventitious shoots and callus of Giza 1 cv. (16.4 and 19.4%) and Giza 21 cv. (15.2 and 19.6%), respectively. Ability of the resistant shoots to produce survived plantlets having roots was investigated during the following study.

Table (23): Evaluating resistance of the micropropagated watermelon shoots obtained from different sources against culture filtrates of *F. oxysporum nevium.*

Cultivar	Source of shoots	Number of tested shoots	Susceptible shoots (dead)		Resistant shoots (survived)	
			No.	%	No.	%
Giza 1	Direct adventitious	500	418	83.6	82	16.4
	Callus	500	403	80.6	97	19.4
	Tetraploid	500	500	100.0	0	0
	Triploid	100	9	9.0	91	91
Giza 21	Direct adventitious	500	424	84.8	76	15.2
	Callus	500	402	80.4	98	19.6
	Tetraploid	500	500	100.0	0	0

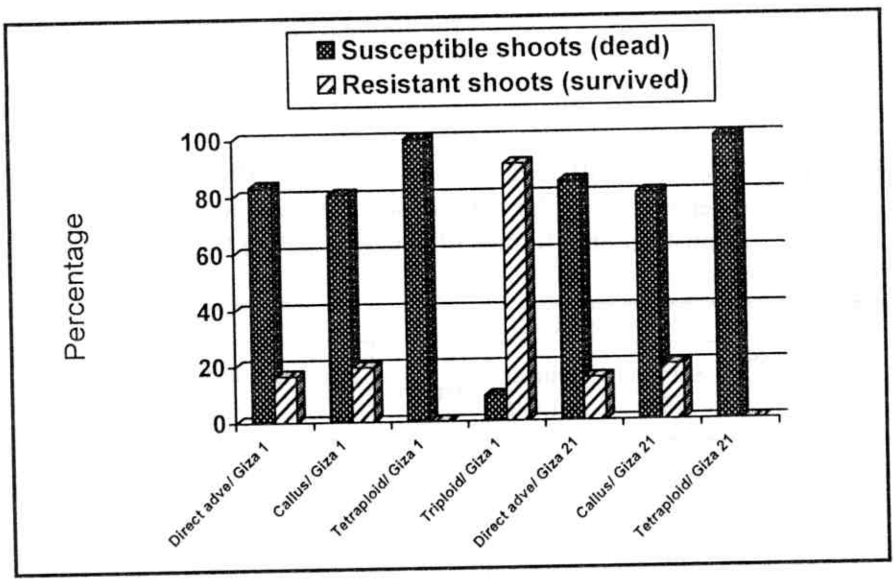


Fig. (21): Evaluation the resistance of the micropropagated watermelon shoots obtained from different sources against culture filtrates of *F. oxysporum nevium.*

III.3. Regeneration of watermelon plantlets showing *in vitro* resistance against culture filtrate of the wilt pathogen:

Data in **Table (24)** reveal that among all tested treatments IBA at 1.5 mg/liter was the most effective since it recorded the highest significant percentage of survived plantlets of both watermelon cultivars (100%) followed by NAA at 0.1 mg/liter in case of Giza 21 only (77.8%). Moreover, IBA at 1.5 mg/liter induced the highest root number (7.0 & 5.3 roots/plantlet) as well as plantlet root length (6.7 & 4.9 cm) for both Giza 1 & Giza 21 cvs., respectively. Most other treatments particularly in Giza 1 cv., showed no significant effect neither on the development of plantlets (55.6-77.8%) nor number of roots (0.3-3.7) compared with the control treatment. The root formation was completely failed on shoots (particularly in Giza 21 cv.) cultured on medium contained the highest concentrations of NAA (1.0 & 1.5 mg/liter).

Table (24): Effect of different concentrations of IBA and NAA on regeneration of the watermelon plantlets showing resistance against culture filtrate of the wilt pathogen (*F. oxysporum nevium*).

Treatment & Conc. (mg/l)		cv. Giza 1			cv. Giza 21		
		Survival (%)	Number of roots	Root length (cm)	Survival (%)	Number of roots	Root length (cm)
IBA	0.0	66.7	1.2	3.7	55.6	1.0	3.6
	0.1	77.8	2.0	3.6	66.7	0.7	3.2
	0.5	55.6	2.7	2.2	44.4	3.3	2.0
	1.0	66.7	3.7	4.0	55.6	4.0	2.7
	1.5	100.0	7.0	6.7	100.0	5.3	4.9
NAA	0.1	66.7	3.0	3.2	77.8	2.3	2.3
	0.5	55.6	2.7	1.7	55.6	1.3	0.7
	1.0	55.6	0.3	1.3	44.4	0.0	0.0
	1.5	55.6	0.0	0.0	44.4	0.0	0.0
L.S.D. at 5%		15.48	2.75	1.88	18.92	1.06	0.85

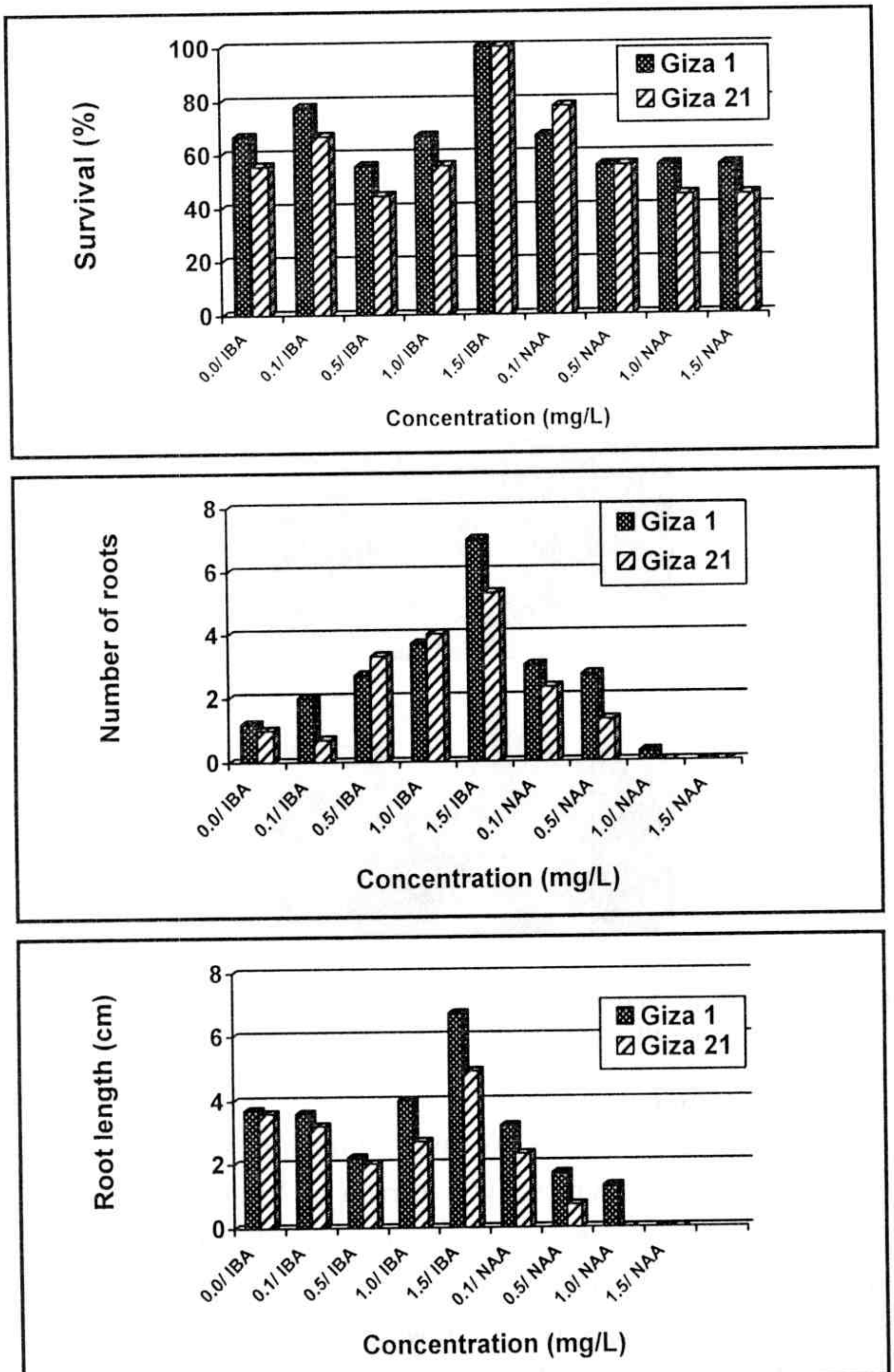


Fig. (22): Effect of different concentrations of IBA and NAA on regeneration of the watermelon plantlets showing resistance against culture filtrate of the wilt pathogen (*F. oxysporum nevium*).

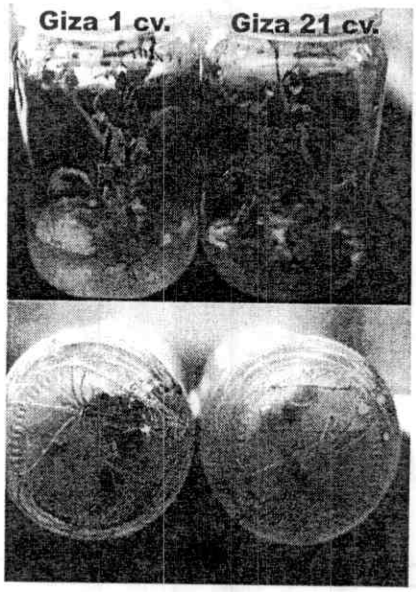


Photo (5): Root formation on watermelon shoots Giza 1 and Giza 21 cultivars after four weeks.

III.4. Acclimatization:

This experiment aimed to study effect of planting media (mixture of sand and peatmoss (v/v) at ratio of 1:0, 1:1, 2:1 or 3:1, respectively) on survival percentage of *in vitro* selected plantlets of Giza 1, Giza 21 and seedless cvs. Data in **Table (25)** show that percentages of plantlet survival grown in mixture of sand and peatmoss at equal volumes (1:1) was significantly higher than those grown in peatmoss alone. The mixture of sand and peatmoss (1:1) recorded 20.0, 16.7 & 16.7% survived plantlets compared with 6.7, 3.3 & 6.7% in peatmoss alone for the watermelon cultivars Giza 1, Giza 21 and triploid (seedless), respectively. Moreover, in the presence of sand, percentages of survival of the 3 cultivars were proportionally and significantly increased with increasing ratio of the peatmoss. Thus, among all tested media, the mixture contained sand and peatmoss at the rate of (1:3 v/v) recorded the highest significant increases in percentages of plantlet survivals of Giza 1 (70.0%), Giza 21 (66.7%), and seedless watermelon (63.3%).

Table (25): Effect of *ex vitro* planting media on survival percentage of *in vitro* selected plantlets.

Tested media		Watermelon cultivar		
Sand	Peatmoss	Giza 1	Giza 21	Seedless
0	1	6.7	3.3	6.7
1	1	20.0	16.7	16.7
1	2	43.3	46.7	33.3
1	3	70.0	66.7	63.3
LSD at 0.05		6.94	13.09	15.11

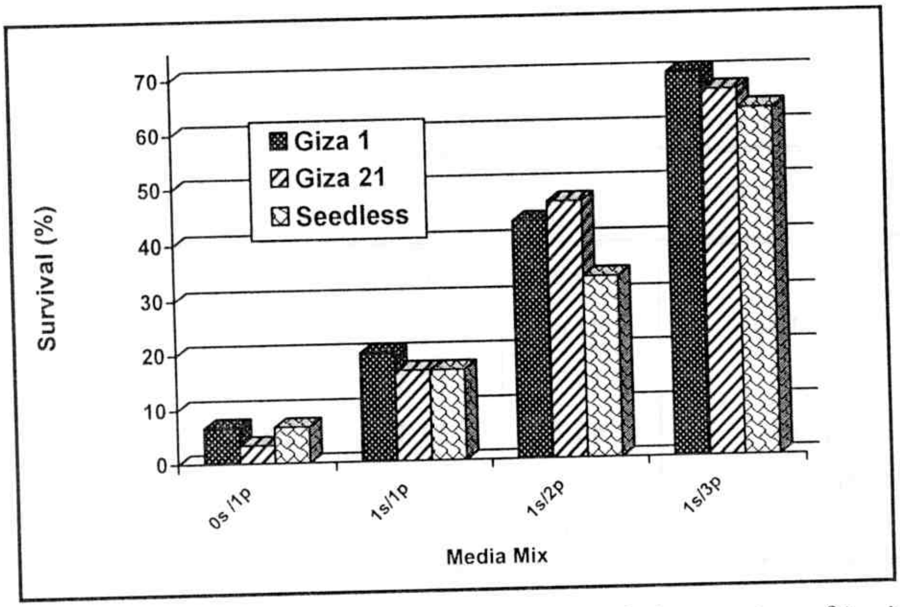


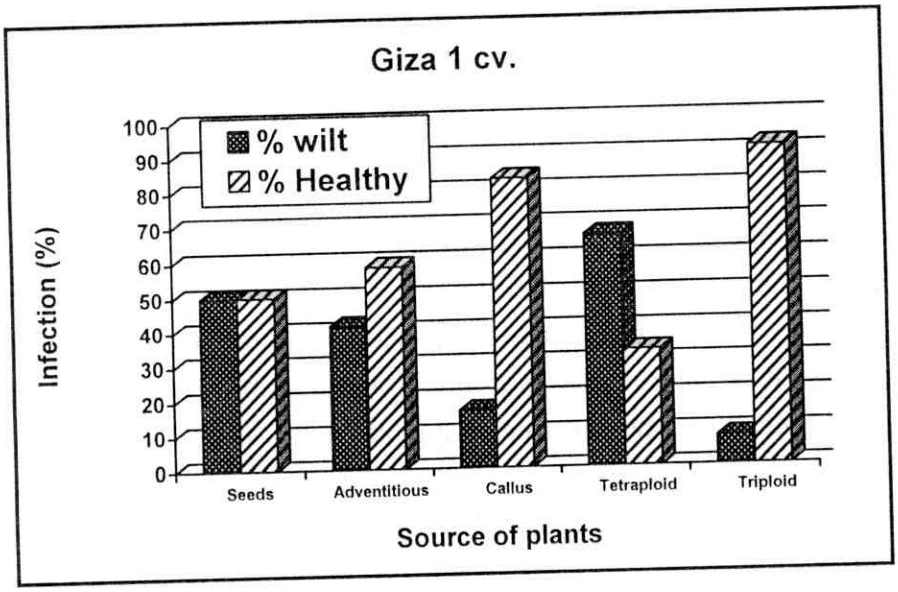
Fig. (23): Effect of *ex vitro* planting media on survival percentage of *in vitro* selected plantlets.

III.5. Effect of greenhouse artificial infection with *F. oxysporum*-isolate No.2 on the *in-vitro* derived watermelon plants:

This experiment was designed to evaluate establishment of resistance in the acclimatized diploid (direct or indirect), triploid and tetraploid watermelon plants of Giza1 and Giza21 cvs. to the artificial infection with *F. oxysporum* isolate No. 2 under greenhouse conditions. The obtained results **(Table, 26)** reveal that only triploid plants of Giza 1 cultivar as well as the diploid ones that were developed from differentiated calluses of both Giza 1 & Giza 21 cultivars were resistant against the artificial infection with the tested isolate of the wilt pathogen. The triploid plants (Giza 1 cv.) showed the lowest percent of wilt (8.3%) followed by callus-plants (16.7%) compared with the control (seed) plants (50.0%). The callus-plants of Giza 21 cv., recorded 25.0% wilt incidence against 58.3% in the control (seed) plants. On contrary, percentage of wilt either in plants developed from cotyledon adventitious shoots or tetraploid plants of both cultivars was not significantly varied when compared with the corresponding percent in their control (seed) plants.

Table (26): Effect of *Fusarium oxysporum* isolates No. 2 on wilt disease incidence on plants of cvs. Giza 1 and Giza 21.

Source of plants	Giza 1		Giza 21	
	% wilt	% Healthy	% wilt	% Healthy
Seeds	50.0	50.0	58.3	41.7
Adventitious	41.6	58.4	50.0	50.0
Callus	16.7	83.3	25.0	75.0
Tetraploid	66.7	33.3	58.3	41.7
Triploid	8.3	91.7	---	---
LSD at 0.05	26.8		12.15	



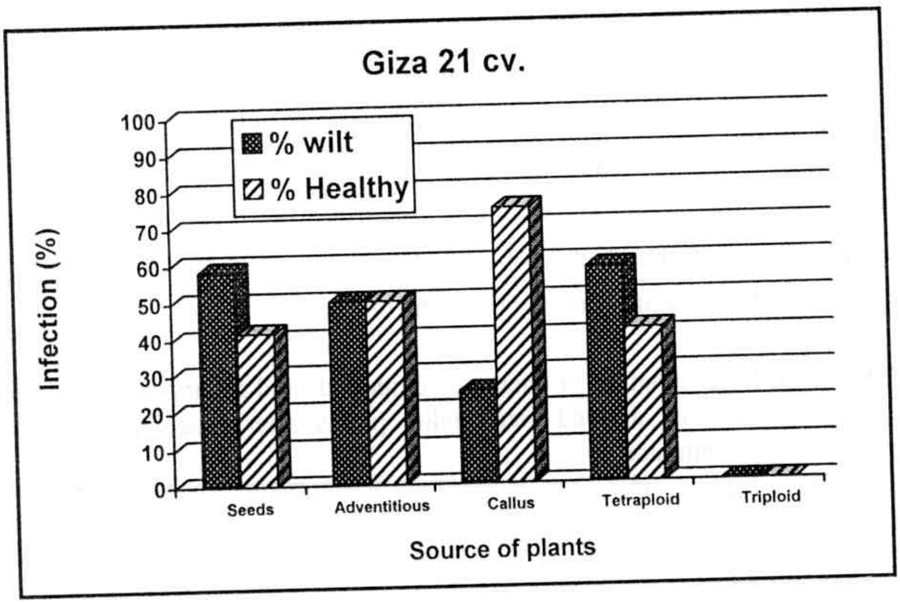


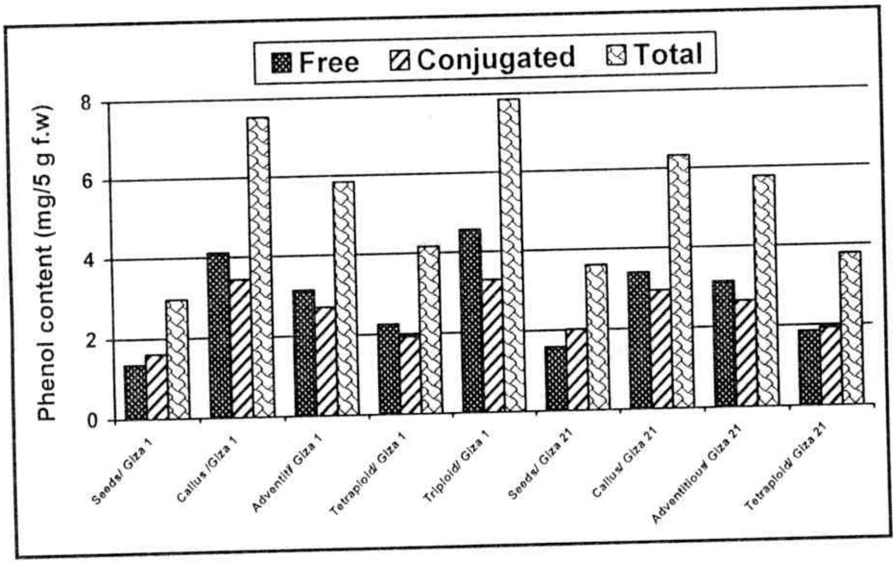
Fig. (24): Effect of *Fusarium oxysporum* isolates No. 2 on wilt disease incidence on plants of cvs. Giza 1 and Giza 21.

III.6. Biochemical changes in the *in-vitro* derived watermelon plants as affected by the artificial infection with *F. oxysporum*-isolate No.2 under greenhouse conditions:

Data in **Table (27)** reveal that the phenols and sugar contents were obviously higher in the *in vitro* derived plants of both watermelon cultivars particularly the triploid and callus derived plantts compared with those grown from seeds (control). Regarding phenols content, the triploid plants (Giza 1 cv.) recorded the highest amounts of free (4.57 mg) and total phenols (7.86 mg) compared with the those derived from seeds (control) which recorded 1.35 & 2.96 mg, respectively. The watermelon diploid plants of Giza 1 and Giza 21 cvs., that were derived from calluses, however, recorded higher free (4.11 & 3.39 mg), conjugated (3.43 & 2.93 mg) and total phenols (7.54 & 6.32 mg) than the tetraploid plants of both cultivars. Compared with the tetraploid and callus plants, plants derived from cotyledon-shoots recorded intermediate amounts of different phenols. Similar trend was also noticed concerning sugars content in the tested plants. The triploid plants (Giza 1 cv.) and plants derived from calluses of Giza 1 and Giza 21 cvs., recorded the highest amounts of reducing, non-reducing and total sugars in comparison with the other investigated plants particularly the control plants.

Table (27): Determination of phenolic compounds and sugars content in the *in vitro* derived watermelon plants after inoculation in the greenhouse.

Cultivar	Source of tested Plants	Phenols (mg/5g f.w)			Sugars (mg/5g f.w)		
		Free	Conjugated	Total	Reducing	Non-reducing	Total
Giza 1	Seeds	1.35	1.61	2.96	4.33	1.16	5.49
	Callus	4.11	3.43	7.54	8.26	1.45	9.71
	Adventitious	3.13	2.70	5.83	3.57	3.47	7.04
	Tetraploid	2.24	1.92	4.16	5.51	0.31	5.82
	Triploid	4.57	3.29	7.86	6.66	1.94	8.60
Giza 21	Seeds	1.59	2.01	3.60	2.48	0.46	2.94
	Callus	3.39	2.93	6.32	9.11	1.68	10.79
	Adventitious	3.12	2.63	5.75	6.70	1.23	7.93
	Tetraploid	1.85	1.92	3.77	3.99	2.20	6.19



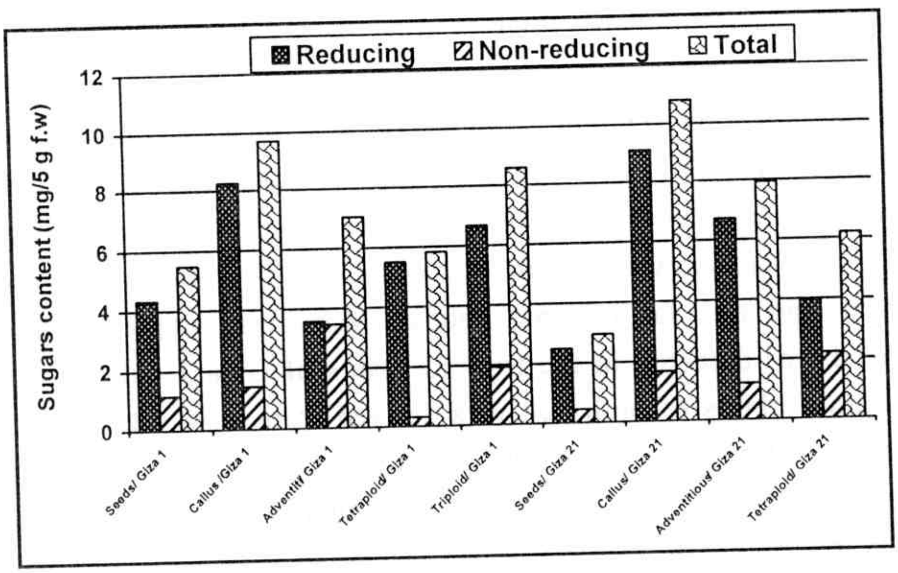


Fig. (25): Determination of phenolic compounds and sugars contents in the *in vitro* derived watermelon plants after inoculation in the greenhouse.

III.8. Isozyme pattern of peroxidase analysis:

Isozyme pattern of soluble peroxidase was studied using double diffusion technique on starch gel electrophoresis. The diploid plants derived from seeds, adventitious buds and callii of Giza 1 and Giza 21 cvs., in addition to the triploid plants of Giza 1 cv., were investigated after plantation in soil artificial infested with isolate 2 of *Fusarium oxysporum* f.sp. *nevium* under greenhouse conditions.

As for peroxidase isozyme, the obtained data **(Fig. 26** and **Photo 6)** show that the tested plants were greatly varied in their peroxidase isozyme pattern. The number of the peroxidase isozyme bands were ranged between 3 to 4 different bands in the tested plants. In this regard, all tested plants were similar concerning the anionic bands since they showed two anionic band varied in their situation (one near and one far anionic bands). On the contrary, the tested plants were greatly varied concerning the cationic bands. In this respect, the diploid control plants of both cultivars (Giza 1 & Giza 21) that derived from seeds as well as diploid plants derived from callus of Giza 21 cultivar showed 2 peroxidase isozyme cationic bands (one near and one far cationic bands). Moreover, single cationic band was detected in the triploid plants of Giza 1 cultivar and diploid plants of both cultivars derived from adventitious buds (near cationic band) while the diploid plants derived from callus of Giza 1 cultivar showed another single cationic band (far band).

Generally, all the tested plants obtained from seeds, adventitious, callus and triploid were similar in cationic peroxidase protein bands but varied in anionic protein bands by using double diffusion - starch gel electrophoresis.

In general, the obtained data showed that Giza-1 and Giza-21 (control) had 1 peroxides protein band more than other tested plants except the plants of 3b (Giza-21) resulted from callus. These control plants are considered susceptible from the previously evaluation. While the other tested plants are considered resistant, from the previous evaluation against *Fusarium oxysporum* f.sp *nevium* filtrates and spores. This means that the susceptible plants had peroxidase bands more than the resistant ones.

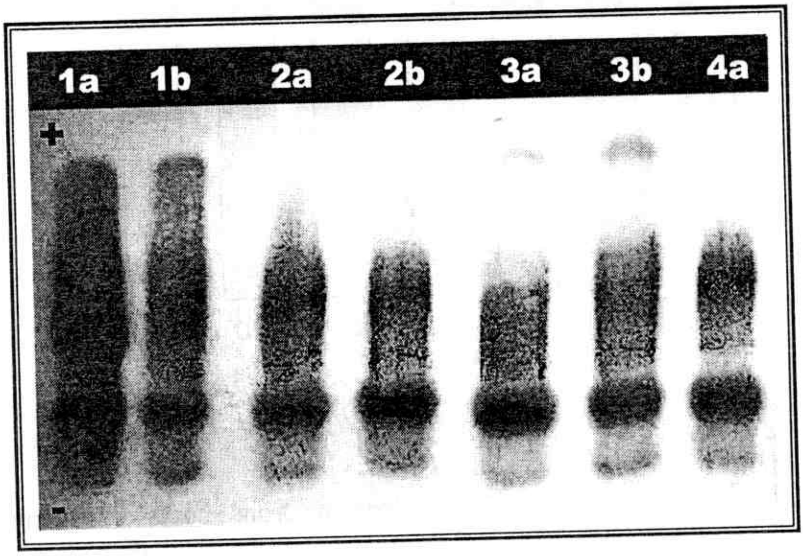


Photo (6): Peroxidase isozyme of watermelon cultivar Giza 1 (a) and Giza 21 (b) at 4 weeks after planting in soil infested with *F. oxysporum nevium* under greenhouse conditions. (1-Control, 2-adventitious shoot, 3-Shoots (callus) and 4- triploid shoots).

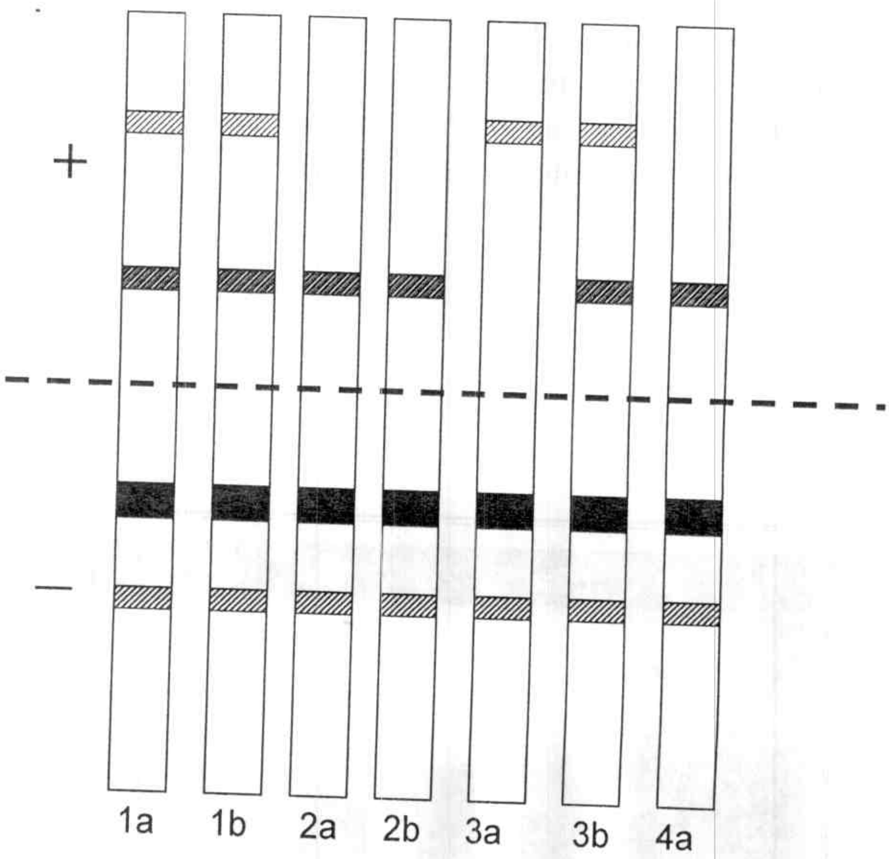


Fig. (26): Zymograme of peroxidase isozyme from shoots of watermelon cultivar Giza 1 (a) and Giza 21 (b) at 4 weeks after planting in soil infested with *F. oxysporum nevium* under greenhouse conditions. (1-Control, 2-adventitious shoot, 3-Shoots (callus) and 4- triploid shoots).

III.8. Evaluation of diploid, triploid and tetraploid watermelon plants to Fusarium wilt under field conditions:

This experiment aimed to evaluate susceptibility of triploid and tetraploid watermelon plants against infection with wilt disease and their yield productivity under field conditions. The local watermelon cultivars Giza 1 and Giza 21 were used for comparison. As for reaction against infection with wilt disease, the obtained data **(Table, 28)** proved that the triploid plants were the most resistant, nearly immune, (0.0% infection) whereas the tetraploid ones were the most susceptible (70.0% infection). However, the local watermelon cultivars Giza 1 and Giza 21 showed intermediate reactions, the first was more resistant (20.0% infection) than the latter one (30.0% infection).

Concerning yield production, data (Table, 28) reveal that the local cultivar Giza 1, however, produced the highest yield/feddan and number of fruits/row followed by the local cultivar Giza 21, then triploid and tetraploid plants, respectively. The average fruit weight and total soluble sugars "TSS" seemed to be correlated positively with the degree of resistance against wilt infection. The most resistant triploid plants produced the highest fruit weight (3.8Kg) and TSS value (13.4%) followed by the local cultivars Giza 1 and Giza 21, respectively. The bulb was more sweetish in Giza 21 and triploid fruits (taste 9) than Giza 1 (taste 8). The most susceptible tetraploid plants, however, produced the lowest yield (1.4 Ton/feddan), number of fruits/row (12), average fruit weight (1.1Kg), taste (7) and TSS (11.8%). **(Photo, 6).**

Table (28): Evaluation of watermelon diploid (Giza 1 and Giza 21), triploid and tetraploid (Giza 1) cultivars against natural infection with Fusarium wilt and some fruit and yield characters under field conditions.

Material	Wilt incidence %	Yield (kg/plant)	Fruit characters				
			No. of fruits /plant	Aver. weight (kg)	Shape index	Taste 1-10	TSS
Giza 1	20	9.8	2.8	3.5	0.95	8	11.9
Giza 21	30	10.3	3.2	3.2	1.00	9	12.8
Triploid	00	9.1	2.4	3.8	1.08	9	13.4
Tetraploid	70	1.3	1.2	1.1	0.87	7	11.8
LSD	12.93	1.2	0.43	0.37	0.17		

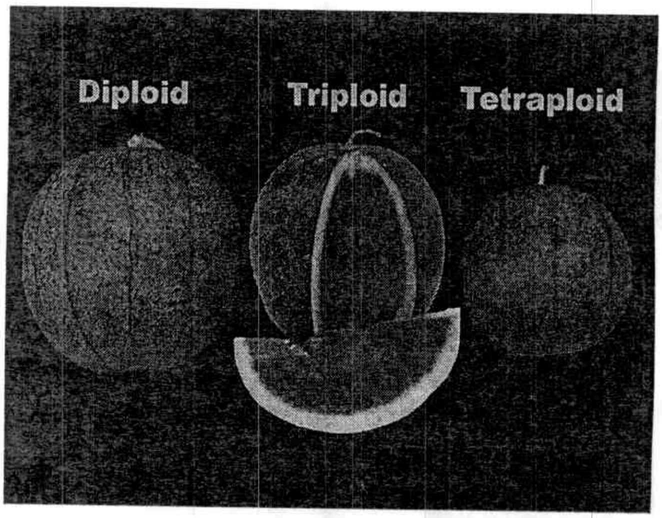


Photo (6): Fruits of diploid, triploid and tetraploid of watermelon cultivar Giza 1.