EXPERIMENTAL RESULTS

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1 -Disease symptoms and diagnosis of the causal viruses:

Healthy looking and naturally infected potato plants showing typical symptoms of mild to severe mosaic or down-word curling of leaves, crinkling and necrosis on the vein of down-side of the leaf or up-word leaf roll with stand upright and pale-yellowish, purple or reddish were collected from different potato growing areas to represent the major potato viruses i.e. Potato X virus (PVX), Potato Y virus (PVY) and Potato leaf roll virus (PLRV), respectively. The viruses associated with these specific symptoms in foliage parts of the infected potato plants were verified on indicator host plants and serologically by direct ELISA test. Physical properties of these viruses were accomplished as follow:-

2 -Survey of Potato viruses :

In this study, fresh samples of potato plants looking healthy or exhibited different viral disease symptoms including mild to severe mosaic, vein banding, leaf rolling and stunting of the plant were collected from different locations in Giza, Gharbia and Qalubia locations during summer seasons 1995-1996. The infectional viruses in saps of the collected samples were detected serologically through ELISA technique.

Data presented in **Table (3)** and **Fig. (1)** show that the three potato viruses i.e. PVX, PVY, and PLRV were detected either singly or in combinations in assayed samples of both potato cultivars Spunta and Diamont. Regarding with Spunta cv., the obtained results revealed that infection with PVY, PLRV, and PVY reached to 74.07 %, 65.0 % and 33.33% in locations of Giza, Qalubia and Gharbia, respectively. For Diamont cv., the occurrence percentages of PVX, PVY and PLRV were 34.46, 46.15 and 43.48 %, in the same locations, respectively.

Table (3): Percentage of infection with PVX, PVY and PLRV and their combinations in potato cvs. Spunta and Diamont in different locations in Egypt.

Locations / Viruses	Spunta cv. at			Diamont cv. at		
	Giza (++)	Qalubia	Gharbia	Giza	Qalubia	Gharbia
PVX	7.41	33.33	10.00	24.14	19.23	26.09
PVY	74.07	22.22	10.00	34.46	46.15	43.48
PLRV	11.11	22.22	65.00	10.34	11.54	21.74
PVX – PVY	7.41	22.22	10.00	10.34	11.54	-
PVX – PLRV	-	-	-	6.90	3.85	-
PVY – PLRV	-	-	-	6.90	3.85	4.35
PVX - PVY – PLRV	-	-	5.00	6.90	3.85	4.35

(++) parenthesized number represents number of examined samples.
(-) Not detected.

Regarding the synchronized complex infection with two or three viruses together in the same infected sample, the present data indicated that it seems occurred at relatively lower percentage than the singular infections. In this regard, the complex infection with PVX + PVY was detected in 22.22, 10.0, and 7.41 % of samples of Spunta cv., collected from Qalubia, Gharbia and Giza locations respectively. While the combined infections with PLRV + PVX or PLRV + PVY were never detected on Spunta cv. The compound infection with the three viruses i.e. PVX + PVY + PLRV was detected in 5.0 % of samples obtained from Gharbia location only.

On potato cv. Diamont, PVY-infection was the more popular in the three location. It was reached to 34.46, 46.15 and 43.48 % in Giza, Qalubia and Gharbia locations, respectively. The singular infections with PVX came the next followed by PLRV-infection. Although complex infection by two or three viruses on Diamont cv. was also less than infection with a single virus but it was more

common on that cultivar in all three locations compared with Spunta cv. From these results it could be concluded in general that, the Diamont cultivar was more susceptible to infection with these viruses in combination than Spunta cultivar.

3 -Effect of different size of explant on growth rate and elimination of potato viruses:

In this experiment, different sizes of explant i.e. 0.2, 0.5 and 0.7 mm were excised aseptically from sprouts emerged on tubers of potato cvs. Spunta and Diamont **(Figs., 2 and 3)** previously proved (through ELISA test) to be infected with the three viruses i.e. PVX, PVY, PVY and PLRV. The excised explants were cultured for up to 20 weeks on MS-medium to study effect of their sizes on the *in vitro* relative growth as well as on elimination of potato viruses associated with the original tubers. Percentages of survived plantlets after 20 weeks were also investigated.

3-1. -Effect of size of explant on percentage of survived plantlets:

It is clear from data presented in **Table (4)** that percentage of survived plantlets produced from explants with length of 0.2 mm was greatly higher (70.8%) in Spunta than Diamont (58.3%) cultivars. However, there are no clear differences in percentage of survived plantlets developed from explants with lengths of 0.5 or 0.7 mm in both cultivars. With the largest size of explant i.e. 0.7 mm, the percentage of survivals reached to 83.3% in both Spunta and Diamont cvs.

Table (4): Effect of size of explant on number @ and percentage of survived potato plantlets cultured on MS-medium for 20 weeks.

	Length of explant (mm)					
	0.2		0.5		0.7	
Cultivar	Number	%	Number	%	Number	%
Spunta	17	70.8	17	70.8	20	83.3
Diamont	14	58.3	16	66.7	20	83.3

@ Average of 4 replicates each included 24 explants.

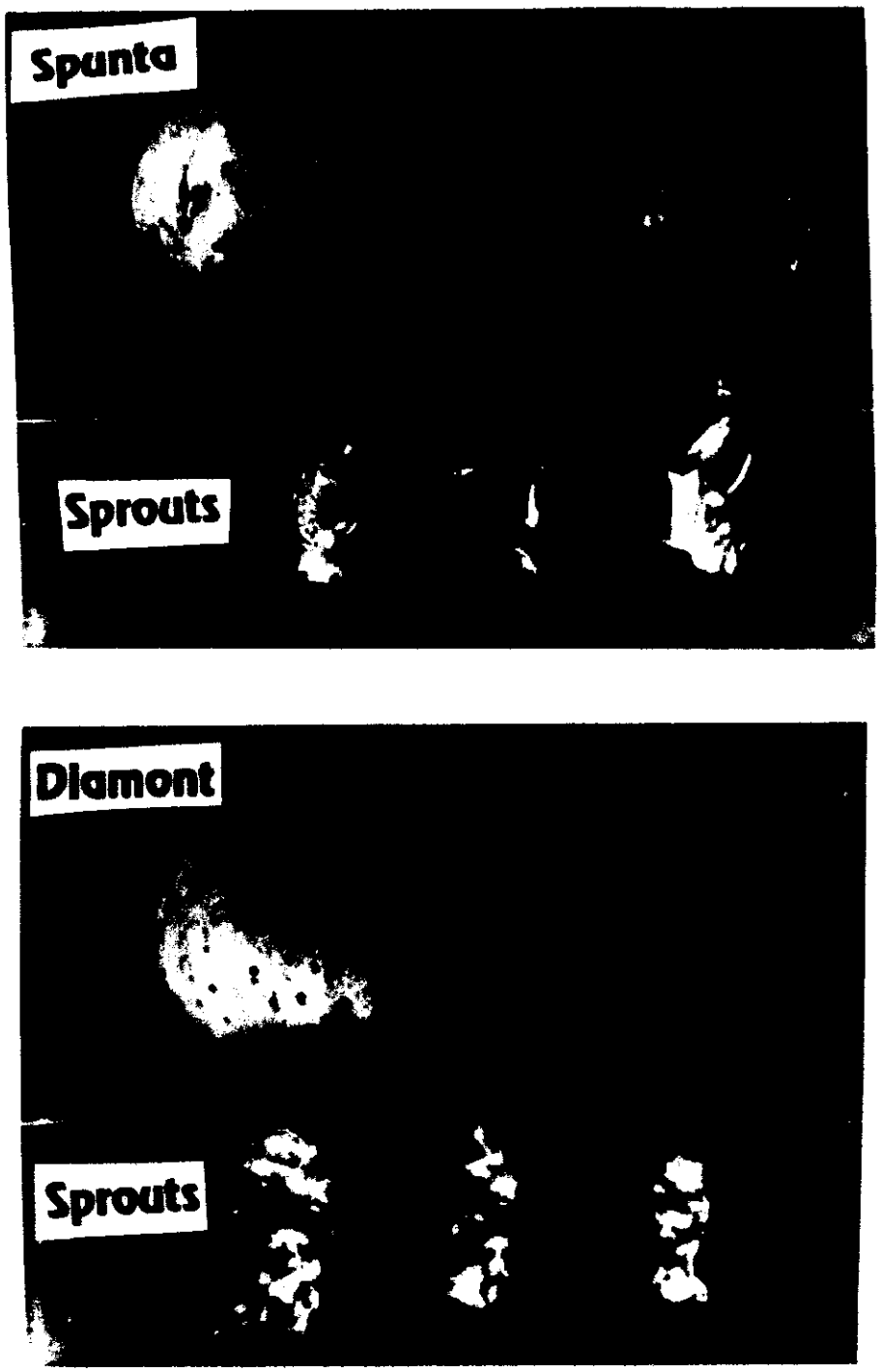


Fig. (2): Sprouted tubers and detached sprouts of two potato cultivars i.e. Spunta and Diamont.

Fig. (3): Excised meristem tips of potato cv., Spunta (explants) at 0.2, 0.5, and 0.7 mm long. (100X)

3-2. -Effect of size of explant on growth rate:

The results obtained in **Table (5)** and **Fig. (4)** showed that, the *in vitro* development and growth rate of both tested potato cvs. I.e. Spunta and Diamont was increased by increasing size of explant from 0.2 to 0.7 mm as well as by prolongation in the incubation time from 4 to 20 weeks. After 20 weeks for example, the relative growth rate reached to 62.5, 79.2 and 89.1%, for 0.2, 0.5 and 0.7 mm explants of Spunta cv., respectively. The corresponding values were 63.64, 65.0 and 83.33 %, respectively in case of Diamont cv.

Table (5): Effect of explant size on percentage of relative growth @ of potato cvs. Spunta and Diamont .in *in vitro*.

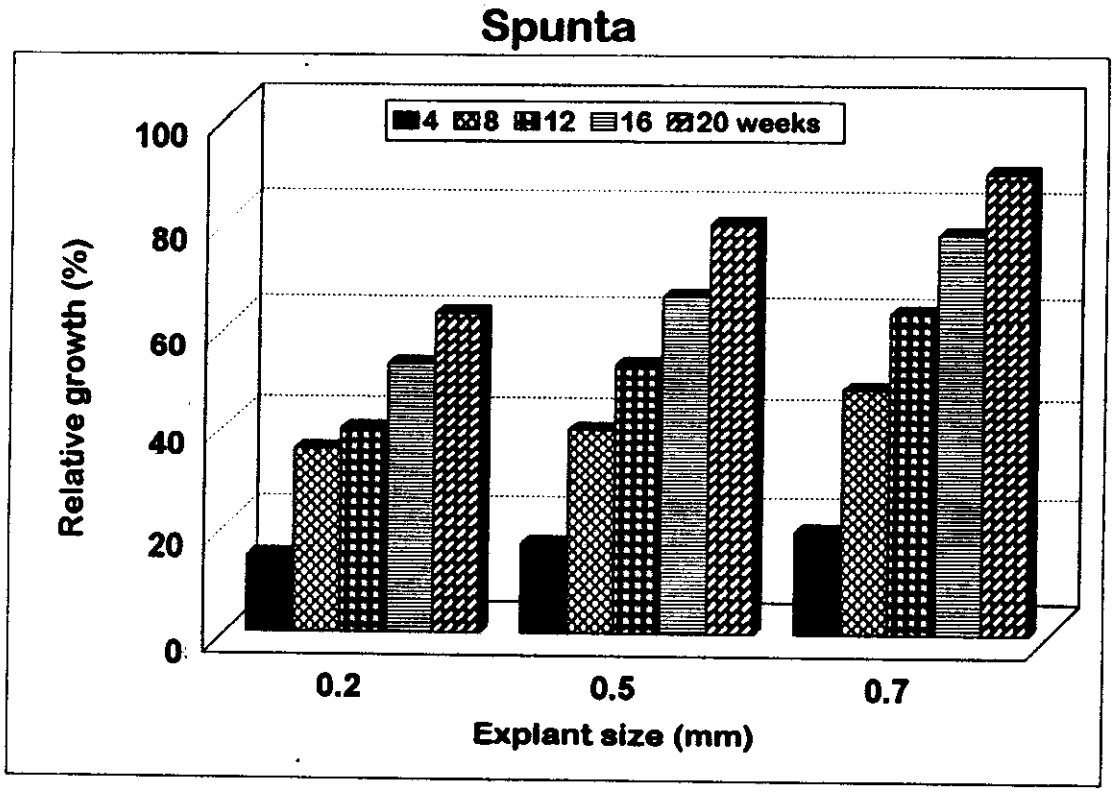
Growth	Size of explant (mm) of Spunta cv				Size of explant (mm) of Diamont cv.			
Period	0.2	0.5	0.7	Mean	0.2	0.5	0.7	Mean
4 weeks	14.17	16.67	19.17	16.67	5.45	8.33	11.66	8.48
8 weeks	35.00	39.10	47.50	40.53	20.00	29.09	33.33	27.47
12 weeks	39.17	52.50	62.72	51.46	28.33	40.00	40.00	36.11
16 weeks	52.50	65.80	77.27	65.19	45.45	48.33	48.33	47.37
20 weeks	62.50	79.20	89.10	76.93	63.64	65.00	83.33	70.66
Mean	40.67	50.65	59.15	50.16	32.57	38.15	43.33	38.02

@ based on 0-5 scale suggested by **Klein & Livingston (1983)**

The present data noticed also that the growth rate of Diamont cv. was somewhat slower than that of Spunta cv. at all tested incubation periods. The average mean of relative growth of Diamont cv. was 38.02 % compared with 50.16 % for Spunta cv.

3-3. -Effect of size of explant on elimination of potato viruses :

For studying effect of size of explant on elimination of viruses associated with the original tubers of potato cvs. Spunta and Diamont, five plantlets of 20-weeks old were selected randomly from those developed from each particular explant size and analyzed serologically by using ELISA technique. The



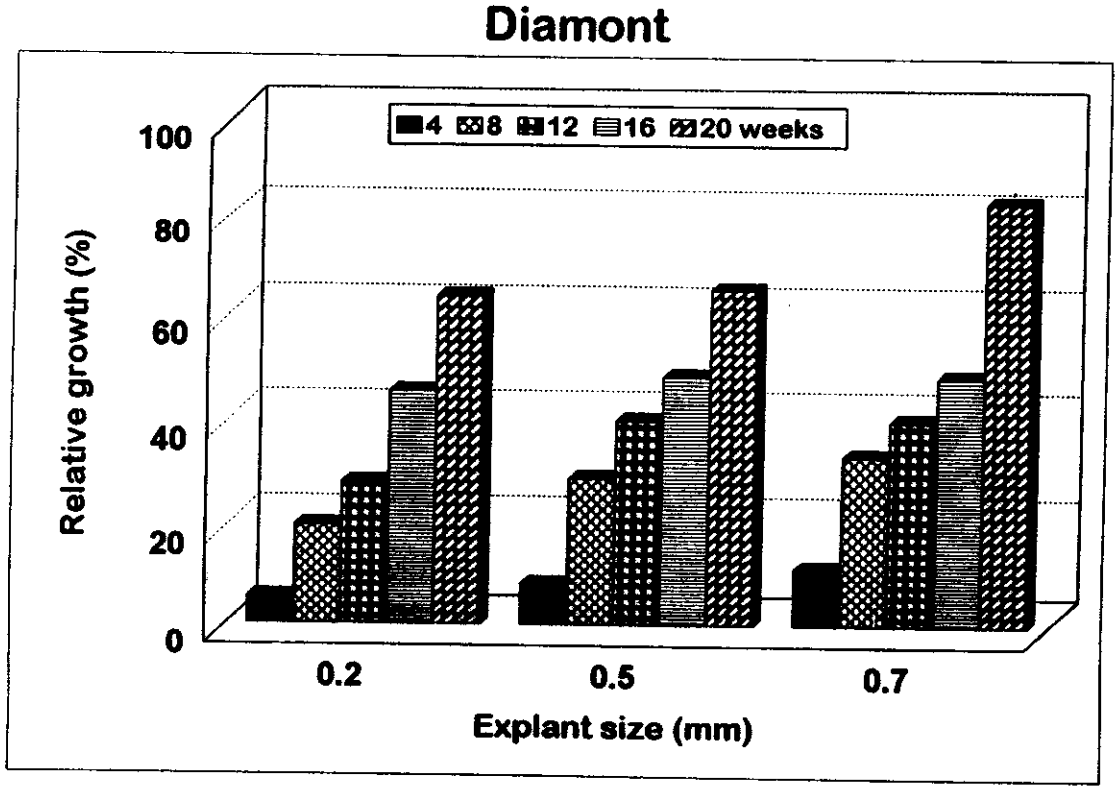


Fig. (4): Effect of different size of explant on growth rate of both Spunta and Diamont *in vitro*.

percentage of infection with potato viruses PVX, PVY and PLRV either singly or in combination as affected by length of explant are presented In **Tables (6)**.

Data in **Table (6)** explored that, the three potato viruses were easily eliminated from plantltes of Diamont cultivar. compared with those of Spunta one. Out of 15 plantltes (for the three sizes of explant for each cultivar) 7 and 3 plantlets were completely freedom from the three viruses in Diamont and Spunta cultivars, respectively. As for explants with 0.2 and 0.5 mm lengths, the same data proved that, 3/5 (60%) and 4/5 (80%) of the plantltes assessed for Diamont cultivar. and 2/5 (40%) and 1/5 (20%) of the plantltes assessed for Spunta cultivar were virus-free, respectively

Table (6): Effect of explant size on number @ and "percentage" of virus-free or virus-infected plantlets of potato cvs. Spunta and Diamont developed from different size of explant.

Detected viruses	Size of explant (mm) of Spunta cv.			Size of explant (mm) of Diamont cv.		
	0.2	0.5	0.7	0.2	0.5	0.7
PVX	0 (0%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)	0 (0%)
PVY	3 (60%)	1 (20%)	1 (20%)	0 (0%)	1 (20%)	5 (100%)
PLRV	0 (0%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)
PVX + PVY	0 (0%)	0 (0%)	2 (40%)	0 (0%)	0 (0%)	0 (0%)
PVX + PLRV	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
PVY + PLRV	0 (0%)	3 (60%)	1 (20%)	1 (20%)	0 (0%)	0 (0%)
PVX + PVY + PLRV	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Virus-free	2 (40%)	1 (20%)	0 (0%)	3 (60%)	4 (80%)	0 (0%)
Total	5(100%)	5(100%)	5(100%)	5(100%)	5(100%)	5(100%)

@ based on 5 plantlets assessed by ELISA for each explant size.

The same data revealed also that the combined infections with PVX + PLRV as well as PVX + PVY + PLRV were completely absent (0.0%) as it was never detected in all tested sizes of explant in both tested potato cvs. The double infections with PVX + PVY and PVY + PLRV as affected by size of explant were less observed in Diamont cv. compared with Spunta cv. It is

surprising that PVY + PLRV- infection was detected only in 1/5 (20%) of plantlets originated from 0.2 mm explant of Diamont while it was noticed in 3/5 (60%) and 1/5 (20%) of plantlets developed from 0.5 and 0.7 mm explants and completely absent in those originated from 0.2 mm explant of Spunta cv. About difficulty of elimination of a singular infection, the present data showed that PVY-infection was more resist as it was detected in 5/15 and 6/15 of plantletes of Spunta and Diamont cvs., respectively. Each of PVX- and PLRV- infection were detected in only 1/15 of plantltes of these cvs., respectively.

3-4. -Effect of virus status of potato plantlets developed through only tissue culture technique alone on production of microtubers:

As for potato cv. Spunta, the data in **Table (7)** illustrated tat the number of microtubers produced by virus-free plantlets and plantlets still infected by PVX alone or PVX + PVY (all developed from 0.5 mm long explants) were significantly equal. However, all other tested characters i.e, total weight and volume of harvested microtubers and average weight and volume of individual microtuber were significantly higher in virus-free plantlets compared with those infected with PVX or PVX+ PVY **(Fig., 5)**. In case of plantlets infected with PVY alone, the average weight of single microtuber was significantly higher than that of virus-free plantlets and this could be attributed to lower number of microtubers produced by PVY-infected plantlets compared with the virus-free ones. The same data shows also the highest significant reduction in all above mentioned tested characters was produced by Spunta-plantlets still infected with PVY alone or combined with PLRV. It could be noticed that the total number, weight of the yielded microtubers, total volume of microtubers and average volume of a single microtuber were reduced by 50, 26.9, 60.8 and 22.5 %, respectively. In case of

Table (7): Comparison between *in vitro* microtuber production of the ELISA tested plantlets of potato cv. Spunta virus-free or infected with PVX, PVY and/or PLRV singly or in combination.

Virus-Status	Total No. of microtuber per 5 plantlets	Total Wt. of microtuber in g per 5 plantlets	Average Wt. of a single microtuber in g	Total Volume in ml.of microtuber per 5 plantlets	Average Volume in ml.of a single microtuber
PVX	14.8	3.64	2.459	3.50	0.24
PVY	8.0	2.91	3.638	2.50	0.31
PLRV	-- @	--	--	--	--
PVX + PVY	14.8	2.97	2.007	3.00	0.20
PVX + PLRV	--	--	--	--	--
PVY + PLRV	8.5	2.36	2.776	2.18	0.26
PVX + PVY + PLRV	--	--	--	--	--
Virus-free	16.0	3.98	2.489	6.38	0.40
L.S.D., at 0.05	1.00	0.32	0.291	0.80	0.03

@ -- = Cases of virus status not detected by ELISA test.

Table (8): Comparison between *in vitro* microtuber production of the ELISA tested plantlets of potato cv. Diamont virus-free or infected with PVX, PVY and/or PLRV singly or in combination.

Virus-Status	Total No. of microtuber per 5 plantlets	Total Wt. of microtuber in g per 5 plantlets	Average Wt. of a single microtuber in g	Total Volume in ml.of microtuber per 5 plantlets	Average Volume in ml.of a single microtuber
PVX	-- @	--	--	--	--
PVY	8.0	1.88	2.350	1.95	0.24
PLRV	6.3	2.21	3.508	2.50	0.40
PVX + PVY	--	--	--	--	--
PVX + PLRV	--	--	--	--	--
PVY + PLRV	5.6	2.05	3.661	1.47	0.26
PVX + PVY + PLRV	--	--	--	--	--
Virus-free	9.0	2.65	2.944	2.02	0.22
L.S.D., at 0.05	3.33	N.S.	N.S.	N.S.	N.S.

@ – = Cases of virus status not detected by ELISA test.

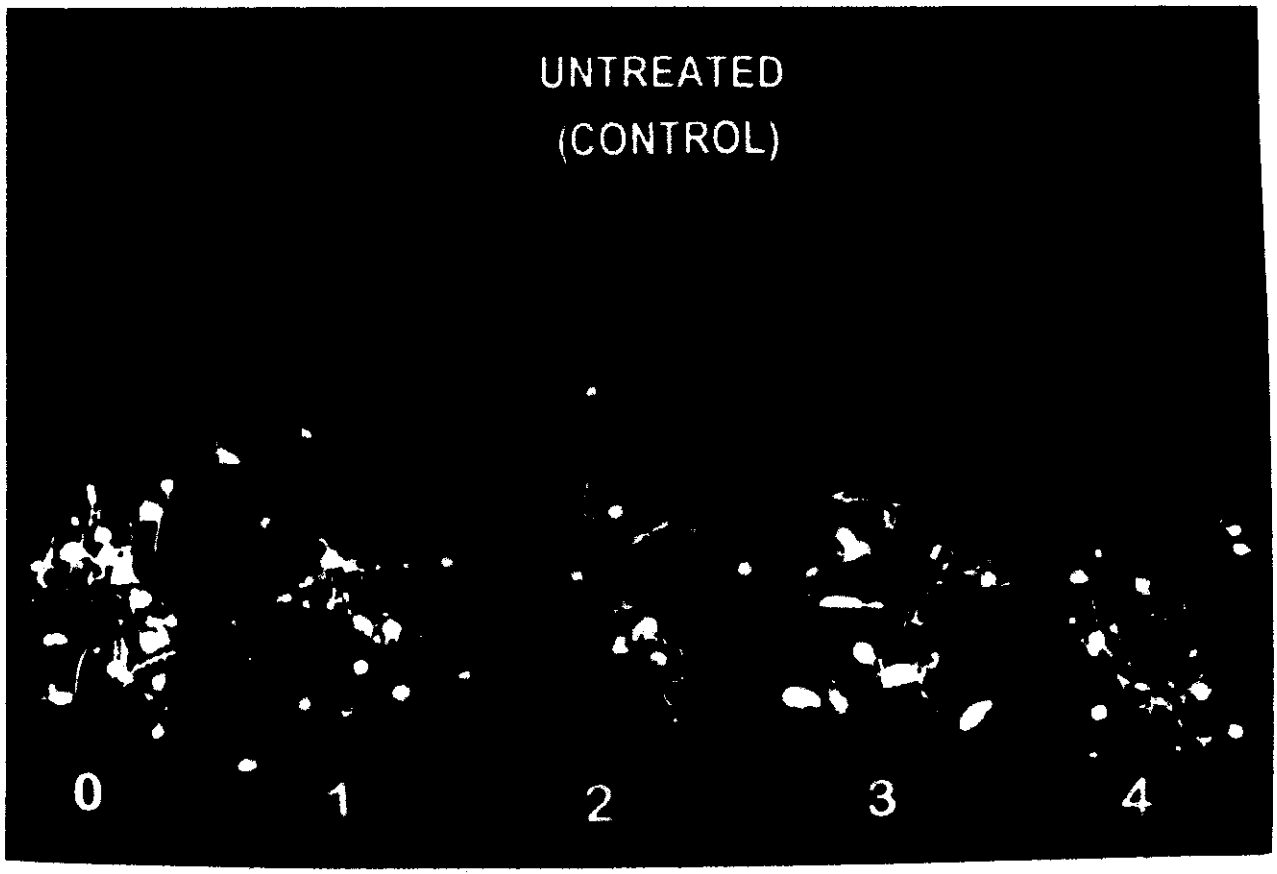


Fig. (5): Microtubers production of Spunta cv., plantlets virus-free (0) or still infected with PVX (1), PVY (2), PVX+PVY (3) or PVY+PLRV (4), all developed from 0.5 mm long explant without any additional treatment.

plantlets infected with PVY + PLRV, these characters reduced by 46.9, 40.7, 53, and 50 %, respectively compared with the virus-free plantlets.

In case of potato cv., Diamont, the data tabulated in **Table (8)** indicated that, the number of harvested microtubers was the only character which was affected significantly by viral infection. In this regard, combined infection with PVY + PLRV reduced the total number of microtubers by 37.8 % followed by PLRV-infection (30 %) and PVY-infection (11 %). Regardless significancy, it could be noticed that infection with PVY was accountable for the highest relative reduction in total weight of microtubers followed by combined infection with PVY + PLRV and PLRV alone, respectively. The latter two infections increased average weight of a single microtuber while PVY-infection decreased it compared with virus-free case. Relative increase in total volume of harvested microtubers was noticed in case of Infection with PLRV alone while infections with PVY alone or combined with PLRV were relatively increased it.

4 -Effect of combination between Virazole treatment and size of explant:

In this experiment, Virazole as antiviral agent was incorporated into MS-culture medium at concentrations of 10, 20 and 40 ppm. in addition to control (without Virazole) to study their effects on percentage of survivals, the *in vitro* growth rate of potato microplants developed from explants taken from sprouts of infected tubers of Spunta and Diamont cultivars at lengths of 0.2, 0.5 and 0.7 mm as well as on elimination of potato viruses associated with these parent tubers.

4-1. -Effect of combination between Virazole treatment and size of explant on survivals:

It is clear from data presented in **Table (9)** and **Fig. (6)** that percentage of survived plantlets were gradually decreased with increasing concentration of Virazole up to 40 ppm. especially with potato cultivar Spunta. As for the latter cultivar, the percentages of survivals at 10 ppm., were 66.0, 79.0, and 79.0% for

explants of 0.2, 0.5 and 0.7 mm, respectively. These percentages decreased to 16.7, 29.2 and 33.3% at 40 ppm, for the three lengths of explant, respectively. With respect to Diamont cultivar, the survival percentage at 40 ppm. of Virazole were 41.7, 50.0 and 50.0 % for explants with 0.2, 0.5 and 0.7 mm, in respective. In control treatment (without Virazole), the percentages of survivals were 70.8, 70.8 and 83.3 % for Spunta cultivar and 58.3, 66.7 and 83.3 % for explants of 0.2, 0.5 and 0.7 mm length, respectively.

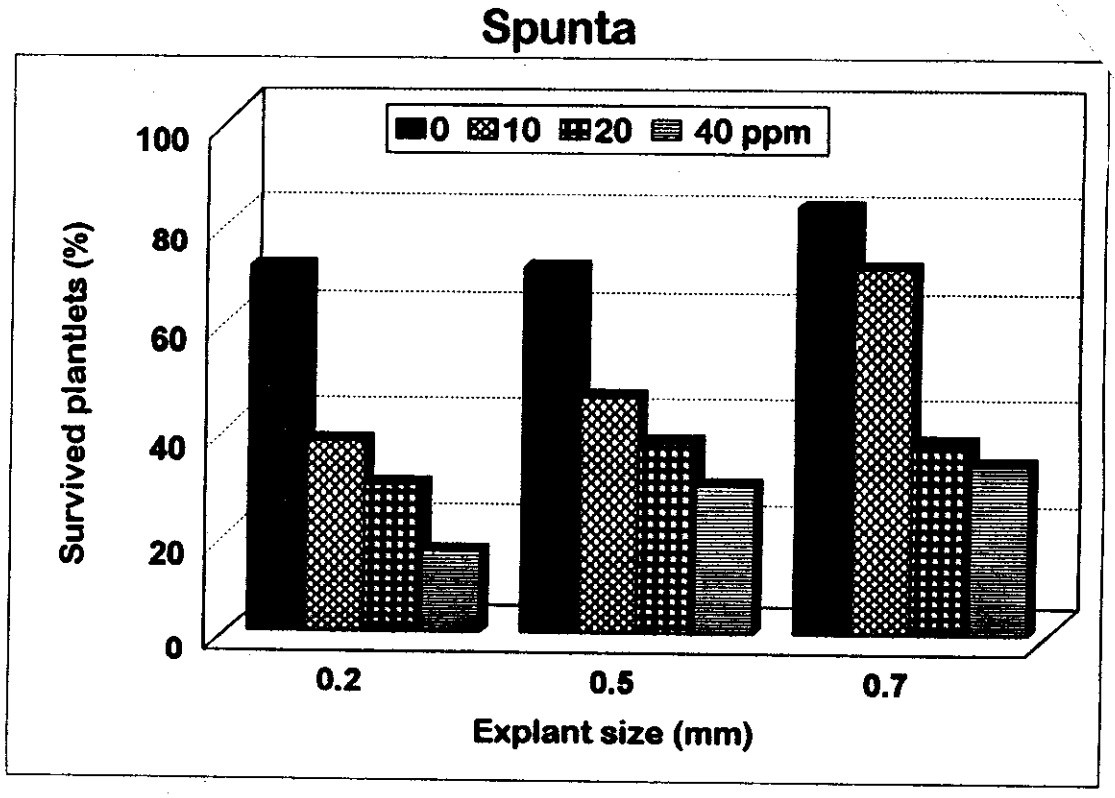
Table (9): Effect of different concentrations of Virazole in MS-culture media on number @ percentage of survived plantlets developed from 0.2, 0.5 and 0.7 mm long explants for potato cultivars Spunta and Diamont.

Cultivar	Virazole conc. in ppm.	0.2 mm		0.5 mm		0.7 mm	
		Number	%	Number	%	Number	%
Spunta	0	17	70.8	17	70.8	20	83.3
	10	9	37.5	11	45.8	17	70.8
	20	7	29.2	9	37.5	9	37.5
	40	4	16.7	7	29.2	8	33.3
Diamont	0	14	58.3	16	66.7	20	83.3
	10	14	58.3	14	58.3	16	66.7
	20	12	50.0	14	58.3	12	50.0
	40	10	41.7	12	50.0	12	50.0

@ Average of 4 replicates each included 24 explants.

4-2. -Effect of combination between Virazole treatment and size of explant on *in vitro* growth rate:

According to data presented in **Tables (10 & 11)** and **Figs. (7 & 8)** it could be noticed that the growth rate of potato explants was successively increased with prolongation in incubation period and it was better in explants grown in control treatment (without Virazole) than those grown in media with Virazole. When the concentration of the Virazole increased the growth rate was consequently decreased. This trend was clear in both potato cultivars i.e. Spunta **(Table, 10)** and Diamont **(Table, 11)**.



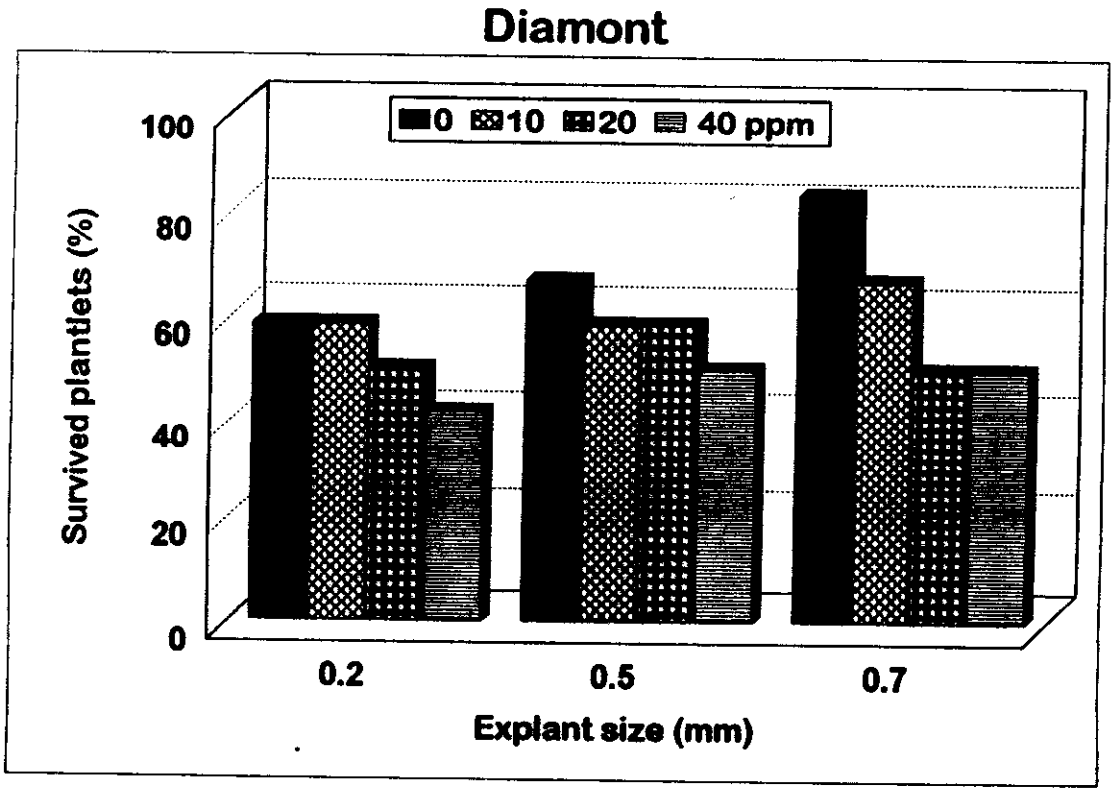


Fig. (6): Effect of different concentrations of Virazole and size of explant on survival percentages of potato cvs. Spunta and Diamont.

Table (10): Effect of Virazole on the growth rate of potato cv. Spunta cultured *in vitro* through 20 weeks.

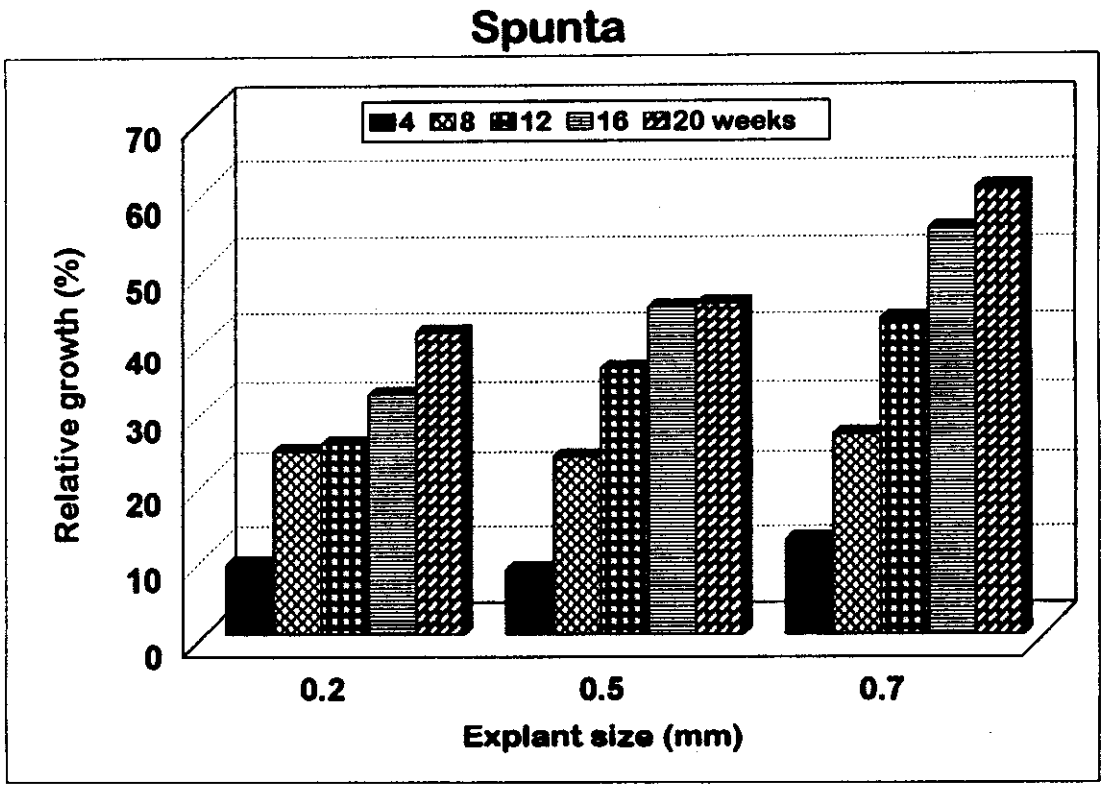
Size of explant (mm)	Concentration (ppm)	Growth Rate (%)				
		4 weeks	8 weeks	12 weeks	16 weeks	20 weeks
0.2	0	14.17	35.00	39.17	52.50	62.50
	10	8.18	40.00	40.91	34.33	45.56
	20	9.09	12.73	22.00	19.13	31.00
	40	4.17	8.71	16.52	22.67	25.71
Mean		8.90	24.11	25.15	32.16	41.19
0.5	0	16.67	47.50	52.50	65.80	79.20
	10	7.83	23.33	35.83	50.43	40.83
	20	5.83	14.78	39.13	36.00	31.00
	40	2.61	7.83	16.52	26.67	30.04
Mean		8.24	23.36	36.00	44.73	45.27
0.7	0	19.17	39.10	62.72	77.27	89.10
	10	15.00	34.17	46.67	58.00	62.00
	20	8.33	16.19	40.00	44.21	46.67
	40	6.09	16.36	23.00	40.00	43.33
Mean		12.15	26.46	43.10	54.87	60.28

@ based on 0-5 scale suggested by **Klein & Livingston (1983)**

Table (11): Effect of Virazole on growth rate of potato cv. Diamont cultured *in vitro* through 20 weeks.

Size of explant (mm)	Concentration (ppm)	Growth Rate (%) after 4 weeks	8 weeks	12 weeks	16 weeks	20 weeks
0.2	0	5.45	29.09	40.00	40.40	63.64
	10	2.00	12.00	22.00	36.00	56.00
	20	1.70	11.70	23.30	30.00	56.70
	40	1.70	10.00	21.70	28.30	38.30
Mean		2.71	15.70	26.75	33.68	53.66
0.5	0	8.33	33.33	40.00	48.33	65.00
	10	2.00	18.30	35.00	48.00	60.00
	20	3.30	12.00	20.00	41.70	58.30
	40	1.70	5.00	10.00	18.30	46.00
Mean		3.83	17.16	26.25	39.08	57.33
0.7	0	11.66	20.00	28.33	48.33	83.33
	10	8.30	15.00	26.70	35.00	70.00
	20	5.00	13.30	21.70	31.70	55.00
	40	5.00	11.70	21.70	31.70	46.70
Mean		7.49	15.00	24.60	36.68	63.76

@ based on 0-5 scale suggested by Klein & Livingston (1983).



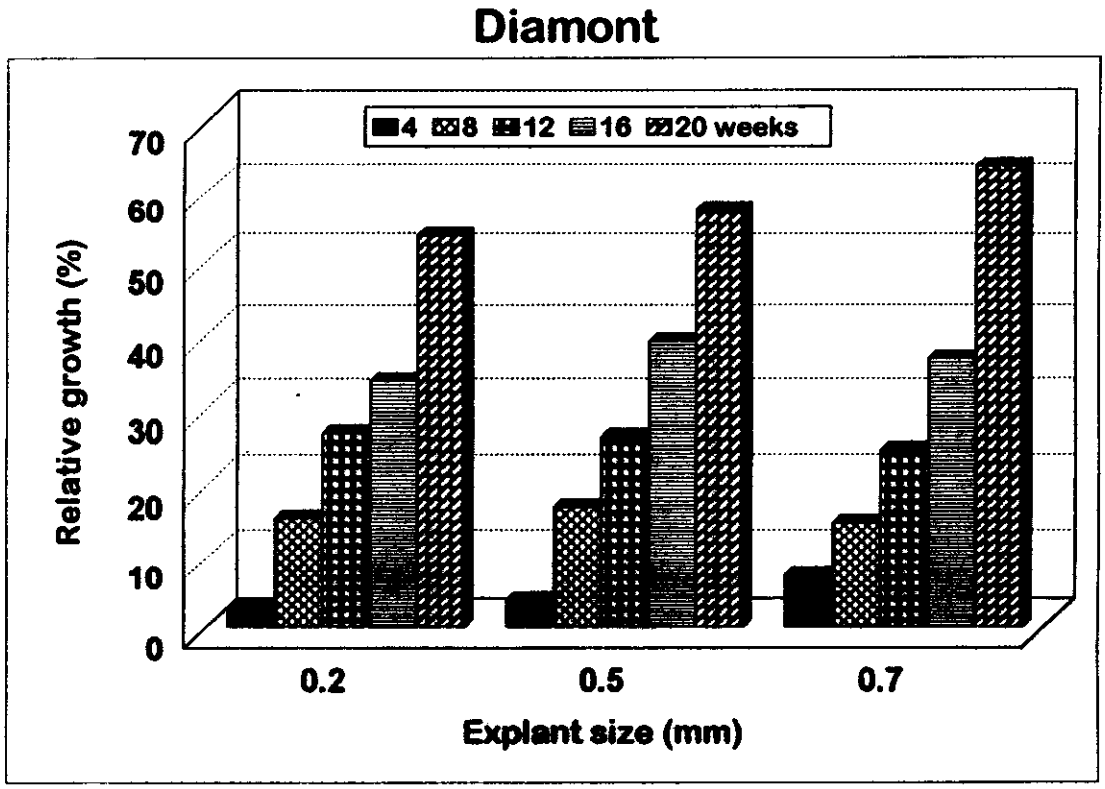


Fig. (7): Effect of different concentrations of Virazole and size of explant on *in vitro* relative growth of potato cv. Diamont.

Fig. (8): Effect of Virazole (VIR) concentrations (ppm) treatment on growth rate of potato plantlets (Spunta cv.) after 1-5 months *in vitro*.

Regardless Virazole treatment, the present data declared also that, the relative growth for any explant length after incubation periods of 4, 8, 12, and 16 weeks, in general was conspicuously higher in Spunta cultivar than Diamont one. However, after 20 weeks, this trend was completely reversed.

4-3. - Effect of Virazole on elimination of viruses

The results about effect of different concentration of Virazole on elimination of viruses are demonstrated in **Tables (12 & 13).** Regarding with production of virus-free plantlets for potato cv. Spunta (**Table, 12**), the present results indicated that, 2/5 , 1/5 and 0/5 virus-free plantlets could be produced by using explants with lengths of 0.2, 0.5 and 0.7 mm, respectively in absence of Virazole (control treatment). In Virazole treatments, It could be noticed that only 1/5 of the plantlets developed from 0.2 mm explant length at Virazole concentrations of 10 and 40 ppm. but not at 20 ppm. were virus-free. No virus-free plantlets could be produced at any given concentration of Virazole when 0.5 mm. explants were used. The most beneficial effect of Virazole was clearly observed when explants with 0.7 mm length were used. With this large size of explants, the number of virus-free plantlets reached to 3/5, 3/5, and 1/5 at concentrations of 10, 20, and 40 ppm., respectively.

Respecting with effect of Virazole on elimination of infection with singular viruses, the same data proved that both PVX- and PLRV-infections were completely eliminated when 0.2 mm explants only were used. With 0.5 mm explants, the PLRV-infection was also eliminated at all tested concentrations of Virazole but PVX-infection was detected in 1/5 of plantlets developed at concentration of 10 ppm. Out of total 20 plantlets developed from explants 0.7 mm length, 4 and 1 were infected with PVX and PLRV, respectively. Regarding with incidence of single infection with PVY, the same data proved that it was detected in 8/20, 9/20 and 2/20 of plantlets developed from 0.2, 0.5 and 0.7 mm explants, respectively. The single infection with PVY seems to be more difficult

to eliminate by Virazole treatments than PVX and PLRV infections. In general, out of total of 60 plantlets tested 5, 19 and 1 were infected with PVX, PVY and PLRV, respectively.

Table (12): Effect of different concentrations of Virazole on elimination of potato viruses using different size of explant in Spunta cultivar.

Size of explant (mm)	Virazole conc. (ppm)	Detected viruses*							Virus-free
		PVX	PVY	PLRV	PVX + PVY	PVX + PLRV	PVY + PLRV	PVX + PVY + PLRV	
0.2	0	-	3	-	-	-	-	-	2
	10	-	3	-	1	-	-	-	1
	20	-	1	-	1	-	3	-	-
	40	-	1	-	-	-	3	-	1
Total		-	8	-	-	-	6	-	4
0.5	0	-	1	-	-	-	3	-	1
	10	1	2	-	2	-	-	-	-
	20	-	4	-	-	-	1	-	-
	40	-	2	-	1	-	1	1	-
Total		1	9	-	3	-	5	1	1
0.7	0	1	1	-	2	-	1	-	-
	10	2	-	-	-	-	-	-	3
	20	1	-	1	-	-	-	-	3
	40	-	1	-	1	-	1	1	1
Total		4	2	1	3	-	2	1	7
Grand Total		5	19	1	8	-	13	2	12

* The ELISA test was carried out on 5 plantlets per each treatment.
(-) Not detected.

As for complex infection, the present data proved that applying any concentration of Virazole with any size of explant resulted in complete elimination of the combined infection with PVX + PLRV. In this regard, the combined infection with PVY + PLRV was occurred more frequently, especially in case of 0.2 and 0.5 mm explants, than infection with both PVX + PVY viruses. The combined infection with the three concerned viruses i.e. PVX + PVY + PLRV

was detected only in 1/5 of the plantlets developed from 0.5 and 0.7 mm explants at 40 ppm concentration of Virazole. In general, out of total of 60 plantlets tested 8, 0, 13, and 1 were infected with PVX + PVY, PVX + PLRV, PVY + PLRV and PVX + PVY + PLRV, respectively.

Table (13): Effect of different concentrations of Virazole on elimination of potato viruses using different size of explant in Diamont cultivar

Size of explant (mm)	Virazole conc. (ppm)	Detected viruses*							Virus-free
		PVX	PVY	PLRV	PVX + PVY	PVX + PLRV	PVY + PLRV	PVX + PVY + PLRV	
0.2	0	-	-	1	-	-	1	-	3
	10	1	1	-	-	-	-	-	3
	20	-	2	-	-	-	-	-	3
	40	-	-	-	-	2	-	-	3
Total		1	3	-	-	2	1	-	12
0.5	0	-	1	-	-	-	-	-	4
	10	-	-	-	-	-	-	-	5
	20	-	2	-	-	-	-	-	3
	40	-	3	1	-	-	-	-	1
Total		-	6	1	-	-	-	-	13
0.7	0	-	5	-	-	-	-	-	-
	10	-	1	-	-	-	-	-	4
	20	-	1	1	-	-	-	-	3
	40	-	1	-	-	-	-		4
Total		-	8	-	-	-	-	-	11
Grand Total		1	17	3	-	2	1	-	36

* The ELISA test was carried out on 5 plantlets per each treatment.
(-) Not detected

As for potato cv. Diamont, the obtained results **(Table, 13)** were nearly similar to that mentioned above especially with effect of Virazole and explant size on incidence and frequency of singular infections with each virus alone. However in case of combined infection, it could be noticed that infections with PVX + PVY was never detected in all treatments and PVY + PLRV-infection was detected only in 1 out of 60 plantlets tested. In contrast, PVX + PLRV-infection

was detected in 2/60 in plantlets of Diamont cv. compared with 0/60 in plantlets of Spunta cv. **(Table, 12).**

In general, the total number of virus-free plantlets was considerably higher in Diamont cv. (36/60) than in Spunta cv. (12/60).

4-4. -Effect of Virazole on microtubers production of virus-free and –infected plantlets *in vitro*:

At 10 ppm of Virazole for potato cv. Spunta, the data illustrated in **Table (14)** and **(Fig., 9-A)** indicated clearly that PVX-infection increased number of harvested microtubers while PVY-infection reduced it significantly compared with those harvested from virus-free plantlets. On other hand, numbers of microtubers from virus-free plantlets and from plantlets infected with PVX + PVY were significantly equal. However, Infection with PVY alone or in combination with PVX reduced total weight of harvested microtubers significantly. PVY-infection reduced it by 38.3 % while PVX + PVY-infection reduced it by 43.7 % compared with virus-free case. Average weight of the individual microtuber was significantly decreased (39.1 %) in case of infection with PVX + PVY compared with virus-free case. At same concentration of Virazole, the present data showed also that, both total volume of harvested microtubers and average volume of the single microtuber were significantly decreased by infection with PVX and PVY each alone or in combination. In general, combined Infection with PVX + PVY caused the highest reduction in both total volume of harvested microtubers (68.7 %) and average volume of a single microtuber (66.7 %) compared with virus-free case.

At concentration of 20 ppm of Virazole, the same data and **(Fig., 9-B)** proved that, the number of harvested microtubers was reduced significantly by all detected virus-status i.e., infections with PVX (57.0 %), PVY (62.9 %), PLRV (32.4 %), PVX + PVY (23.5 %), and PVY + PLRV (55.9 %) compared with virus-free status. Most of the other tested characters of microtubers i.e. total weight

Table (14): *Effect of different concentrations of virazole on the in vitro microtuber production of plantlets of potato cv. Spunta either virus-free or infected with PVX, PVY and/or PLRV singly or in combination.*

Virus-Status	Total No. of microtuber per plantlet @				Total Wt. of microtuber in g per plantlet				Average Wt. of a single microtuber in mg				Total Volume in ml. of microtuber per plantlet				Average Volume in ml.of a single microtuber			
	Control	10 ppm	20 ppm	40 ppm	Control	10 ppm	20 ppm	40 ppm	Control	10 ppm	20 ppm	40 ppm	Control	10 ppm	20 ppm	40 ppm	Control	10 ppm	20 ppm	40 ppm
PVX	14.8	15.8	7.3	--	3.64	3.75	2.27	--	245.9	237.3	311.0	--	3.50	3.75	1.75	--	0.24	0.24	0.28	--
PVY	8.0	10.0	6.3	18.5	2.91	2.56	1.53	3.00	363.8	320.0	242.9	162.2	2.50	2.25	1.63	2.38	0.31	0.23	0.22	0.13
PLRV	-- A	--	11.5	--	--	--	1.79	--	--	--	137.7	--	--	--	1.73	--	--	--	0.15	--
PVX + PVY	14.8	12.0	13.0	10.8	2.97	2.28	1.75	3.11	200.7	154.1	145.8	288.0	3.00	1.88	1.63	2.88	0.20	0.16	0.13	0.27
PVX + PLRV	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
PVY + PLRV	8.5	--	7.5	16.3	2.36	--	1.36	3.31	277.6	--	181.3	203.1	2.18	--	1.43	3.25	0.26	--	0.19	0.20
PVX + PVY + PLRV	--	--	--	17.3	--	--	--	2.57	--	--	--	148.6	--	--	--	2.38	--	--	--	0.14
Virus-free	16.0	12.5	17.0	11.0	3.98	4.05	3.43	3.32	248.9	253.1	201.8	301.8	6.38	6.00	5.25	4.75	0.40	0.48	0.31	0.43
L.S.D., at o,05	1.00	1.10	1.3	1.7	0.32	0.43	0.18	N.S.	29.1	53.0	11.9	37.7	0.80	0.46	0.31	0.95	0.03	0.07	0.01	0.04

@ = *A single plantlet divided into five segments and cultured together in one jar.*
A = Cases of virus status not detected by ELISA test.

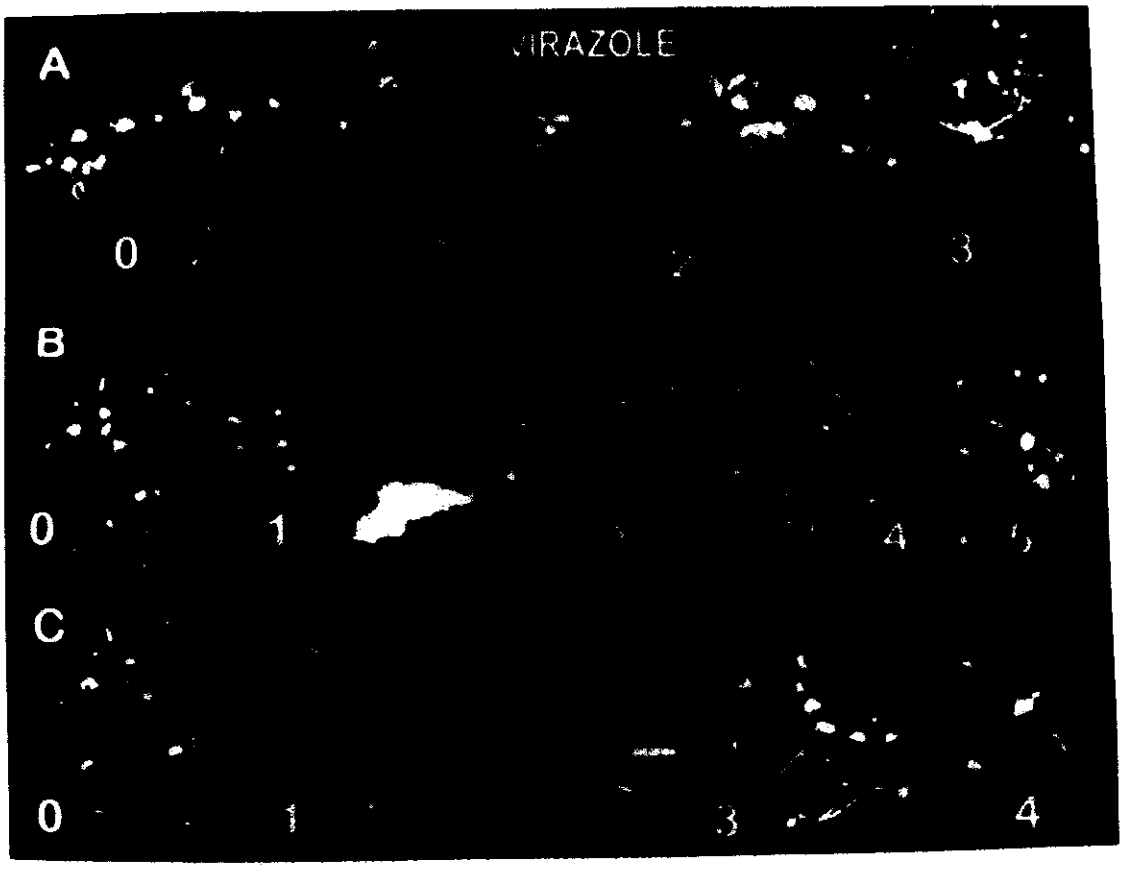


Fig. (9): Effect of Virazole at 10 ppm (A), 20 ppm (B), and 40 ppm (C) on microtubers production of virus-free plantlets (0) as well as those still infected with:
PVX(1-B, 2-A,C), PVY(1-A,C, 2-B), PVY+PVX(3-A,C, 4-B), PLRV(3-B), PVY+PLRV(3-C, 5-B), PVX+PVY+PLRV (4-C).

and volume of harvested microtubers and average volume of a single microtuber were reduced significantly by each one of the detected viral-status. In general, the highest reduction in total weight of microtubers per plantlet was caused by compound infection with PVY + PLRV (60.3 %) followed by infection with PVY alone (55.4). However, combined infection with PVY + PLRV and PVX + PVY caused the highest significant reductions in total volume of microtubers per plant and average volume of a single microtuber i.e. 72.8 % and 58.1 %, respectively.

With respect to 40 ppm of Virazole, the same data in **Table (15)** and **(Fig., 9-C)** indicated that, the total number of microtubers per plantlet was significantly higher in most detected cases of viral-infected plantlets (except PVX + PVY) compared with virus-free plantlets. It could be observed also that total weight of microtubers per plantlet was significantly equal in both virus-free and virus-infected plantlets. The average weight of single microtuber was, however, significantly reduced by 46.3 %, 32.7 and 50.8 % in case of infection with PVY, PVY + PLRV and PVX + PVY + PLRV, respectively. Total volume of microtubers per plantlets was significantly reduced by 49.9 % in case of PVY-infection, 39.4 % in case of PVX + PVY-infection, 31.6 % in case of PVY + OLRV-infection and 49.9 % in case of infection with the three viruses i.e. PVX + PVY + PLRV. As for average volume of a single microtuber, percentages of reduction caused by these viral-infections were 69.8, 37.2, 53.5 and 67.4 %, respectively.

Regarding with potato cv. Diamont, the obtained data **(Table, 15)** proved that, both total number of microtuber per plantlet and average volume of a single microtuber at both 20 and 40 ppm of Virazole concentrations, and total volume of microtubers per plantlet were significantly affected by variety of viral-infections. At concentration of 20 and 40 ppm for PVY-infection and 20 ppm for PLRV-infection, the total number of microtuber per plantlet was significantly higher than the corresponding number of virus-free plantlets. However at concentration of

Table (15): Effect of different concentrations of virazole on the *in vitro* microtuber production of plantlets of potato cv. Diamont virus-free or infected with PVX, PVY and/or PLRV singly or in combination.

Virus-Status	Total No. of microtuber per plantlet @				Total Wt. of microtuber in g per plantlet				Average Wt. of a single microtuber in mg				Total Volume in ml. of microtuber per plantlet				Average Volume in ml.of a single microtuber			
	Control	10 ppm	20 ppm	40 ppm	Control	10 ppm	20 ppm	40 ppm	Control	10 ppm	20 ppm	40 ppm	Control	10 ppm	20 ppm	40 ppm	Control	10 ppm	20 ppm	40 ppm
PVX	– A	12.0	–	–	–	1.85	–	-	–	154.2	–	–	–	1.75	–	–	–	0.15	–	–
PVY	8.0	10.3	12.0	12.8	1.88	1.74	2.38	1.40	235.0	168.9	198.3	116.7	1.95	1.67	1.95	1.62	0.24	0.16	0.16	0.13
PLRV	6.3	–	13.0	–	2.21	–	2.48	–	350.8	–	190.8	–	2.50	–	1.67	–	0.40	–	0.13	–
PVX + PVY	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PVX + PLRV	–	–	–	6.0	–	–	–	1.05	–	–	–	175.0	–	–	–	1.17	–	–	–	0.20
PVY + PLRV	5.6	–	–	–	2.05	–	–	–	366.1	–	–	–	1.47	–	–	–	0.26	–	–	–
PVX + PVY + PLRV	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Virus-free	9.0	9.0	10.0	10.0	2.65	1.74	1.88	1.90	294.4	193.3	188.0	190.0	2.02	2.55	1.87	3.00	0.22	0.28	0.19	0.30
L.S.D., at 0.05	3.33	N.S.	1.75	3.15	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	0.25	N.S.	0.11	0.05	0.06

@ = A single plantlet divided into five segments and cultured together in one jar.

A = Cases of virus status not detected by ELISA test

40 ppm for complex infection with PVY + PLRV, the total number of microtuber per plantlet was significantly reduced by 40.0 % of those produced by virus-free plantlets. On the other hand, total volume of microtubers per plantlet and average volume of individual microtuber in case of treatment with 40 ppm of Virazole were reduced by 46% and 56.7%, in respective, in case of infection with PVY alone and by 61% and 33.3%, in respective, in case of infection with PVX + PLRV compared with virus-free case.

5 - Effect of combination between size of explant and salicylic acid treatment:

In this experiment, salicylic acid as antiviral agent was incorporated into MS-culture medium at concentrations of 10, 20 and 40 ppm. in addition to control (without salicylic acid) to study their effects on percentage of survivals, the *in vitro* growth rate of potato microplants developed from explants taken from sprouts of infected tubers of Spunta and Diamont cultivars at lengths of 0.2, 0.5 and 0.7 mm as well as on elimination of potato viruses associated with these parent tubers.

5-1. - Effect of salicylic acid on survival percentage:

The obtained data in **Table (16)** and **Fig. (10)** proved that there was no clear differences in percentages of survived plantlets of both potato cultivars either developed from explants with length of 0.2 or 0.5 mm. These observations were true in all tested concentrations of salicylic acid. The percentage of survivals was relatively higher at concentration of 10 ppm. than 20 or 40 ppm. of salicylic acid. It was noticed also that percentage of survived plantlets developed from explants with length of 0.7 mm at all tested concentrations of salicylic acid were greatly higher, especially in Diamont cv., than those developed from 0.2 or 0.5 mm explants.

Table (16): Effect of different concentrations of Salicylic acid in MS-culture media on number @ percentage of survived plantlets developed from explants with different lengths (in mm) for potato cultivars Spunta and Diamont.

Cultivar	Salicylic acid conc. In ppm.	Different size of explant (mm)					
		0.2		0.5		0.7	
		Number	%	Number	%	Number	%
Spunta	0	17	70.8	17	70.8	20	83.3
	10	12	50.0	12	50.0	12	50.0
	20	10	41.7	10	41.7	12	50.0
	40	9	37.5	10	41.7	10	41.7
Diamont	0	14	58.3	16	66.7	20	83.3
	10	12	50.0	14	58.3	14	58.3
	20	10	41.7	10	41.7	14	58.3
	40	9	37.5	10	41.7	12	50.0

@ Average of 4 replicates each included 24 explants.

5-2. - Effect of salicylic acid on rate of growth:

The effect of different concentrations of salicylic acid i.e. 0, 10, 20 and 40 ppm on the *in vitro* relative growth of potato plantlets, developed from different sizes of explants of Spunta and Diamont cvs., was studied.

In this regard, the obtained results included in **Tables (17 & 18)** and **Figs. (11 & 12)** revealed that salicylic acid has an inhibition effect on the *in vitro* growth of plantlets of Spunta and Diamont cultivars when compared with control (without acid treatment). This inhibition was increased with increasing concentrations of salicylic acid. Regardless size of explant and concentrations of salicylic acid, the growth rate was increased by prolongation of incubation period. At all incubation periods except the first 4 weeks, the relative growth was clearly high in plantlets of Diamont cv. than Spunta cv

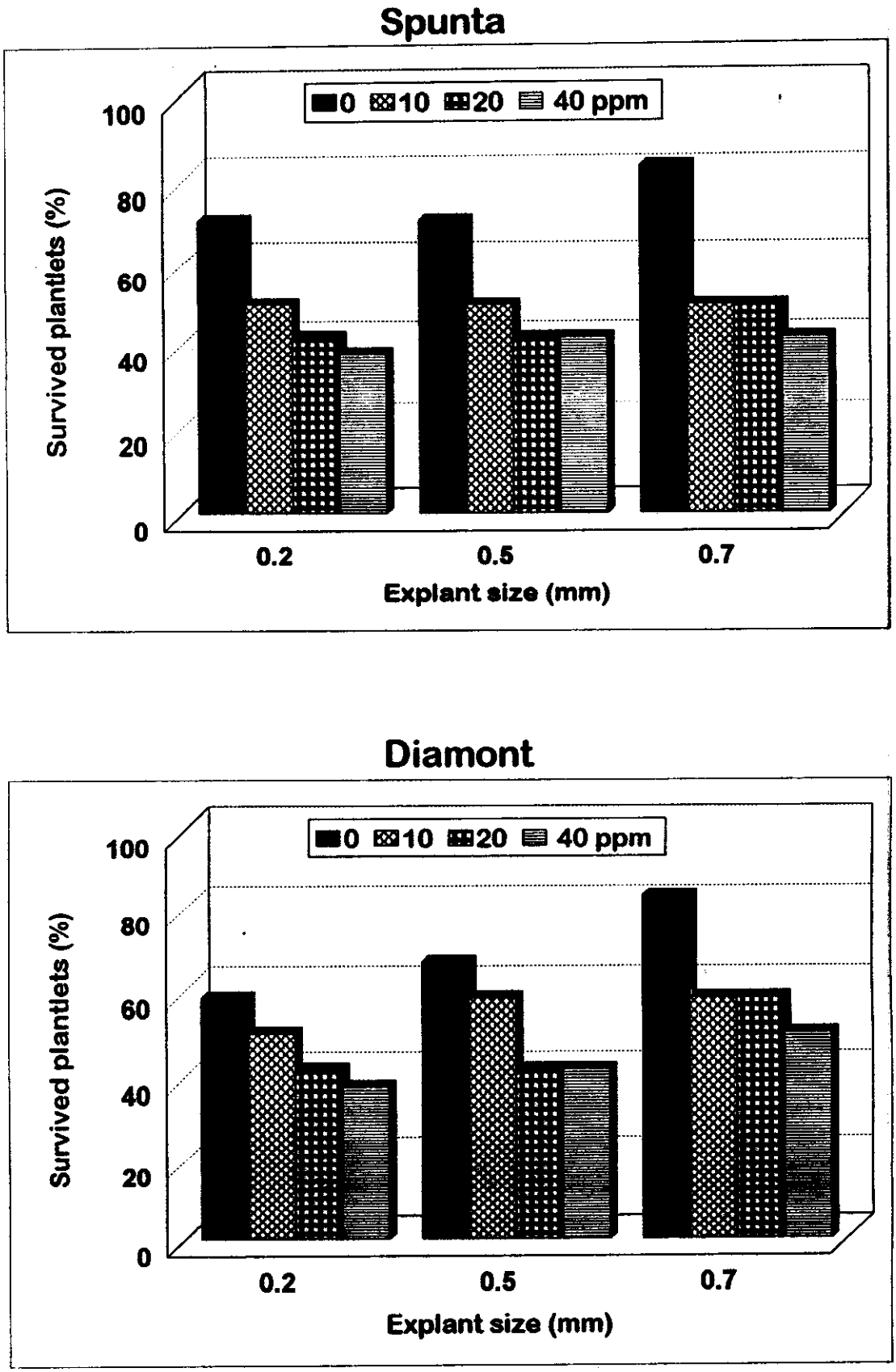


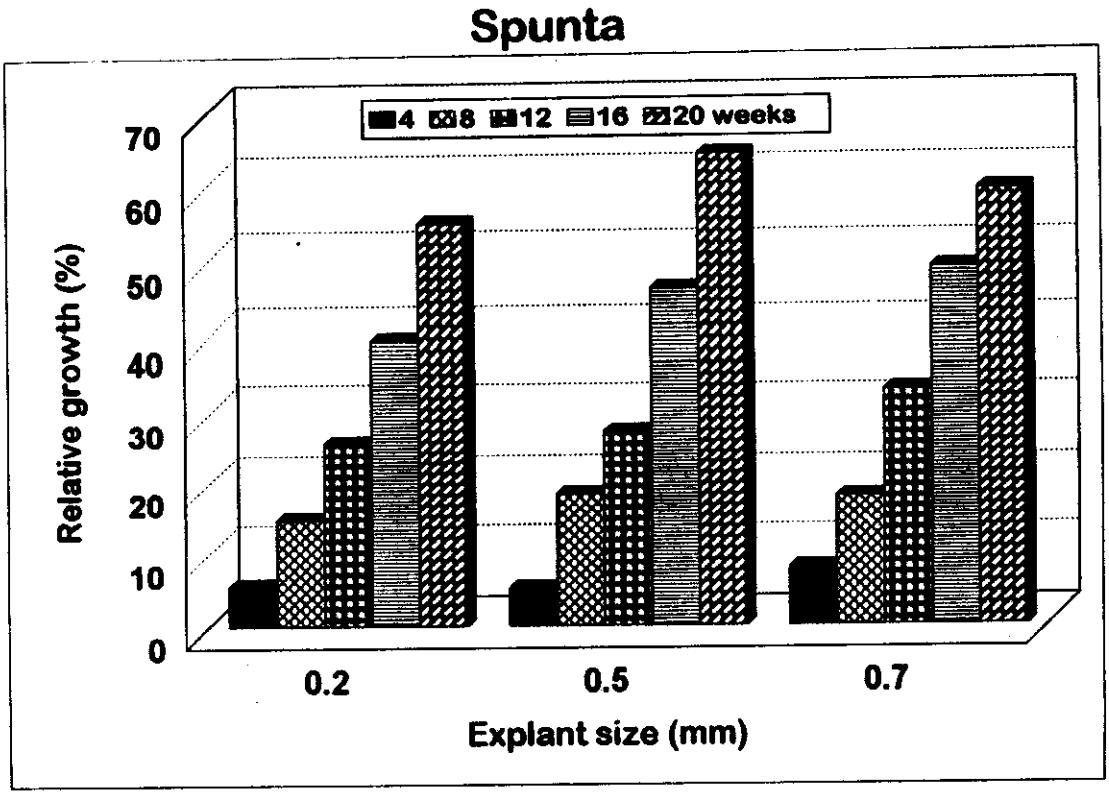
Fig. (10): Effect of different concentrations of Salicylic acid and size of explant on survival percentages of potato cvs. Spunta and Diamont.

Table (17): Effect of salicylic acid on the growth and development (growth rate) of potato cv. Spunta cultured *in vitro* through 20 weeks.

Size of explant (mm)	Concentration (ppm)	Growth Rate (%)				
		4 weeks	8 weeks	12 weeks	16 weeks	20 weeks
0.2	0	14.10	35.00	39.17	52.50	62.50
	15	5.83	11.82	25.00	37.78	56.47
	30	1.00	9.00	21.00	36.67	52.00
	45	1.05	3.00	18.18	31.11	48.57
Mean		5.50	14.71	25.84	39.52	54.89
0.5	0	16.67	47.50	52.50	65.80	79.20
	15	2.00	11.00	21.00	54.12	67.06
	30	2.00	7.00	20.00	33.33	58.75
	45	0.00	8.00	16.25	31.25	51.11
Mean		5.17	18.38	27.44	46.13	64.03
0.7	0	19.17	39.10	62.72	77.27	89.10
	15	5.45	13.64	28.18	40.00	55.00
	30	4.17	10.91	25.00	39.00	50.91
	45	2,75	8.00	16.47	38.82	41.18
Mean		7.89	17.91	33.09	48.77	59.05

Table (18): Effect of salicylic acid on the growth and development (growth rate) of potato cv. Diamont cultured *in vitro* through 20 weeks.

Size of explant (mm)	Concentration (ppm)	Growth Rate (%)				
		4 weeks	8 weeks	12 weeks	16 weeks	20 weeks
0.2	0	5.45	29.09	40.00	45.45	63.64
	10	2.00	10.00	16.00	26.00	42.00
	20	1.70	6.70	11.00	21.00	40.00
	40	1.70	5.00	10.00	18.33	41.70
Mean		2.71	12.70	19.25	27.70	46.84
0.5	0	8.33	20.00	40.00	48.33	65.00
	10	3.33	18.33	25.30	33.30	61.70
	20	3.33	10.00	16.70	23.70	45.00
	40	1.70	6.70	11.70	18.33	23.33
Mean		4.17	13.76	23.35	28.42	48.76
0.7	0	11.66	33.33	28.33	48.33	83.33
	10	6.70	13.33	28.33	38.33	60.00
	20	3.30	10.00	16.70	23.33	60.00
	40	1.70	6.70	13.30	23.33	45.00
Mean		5.84	15.84	21.67	33.33	62.08



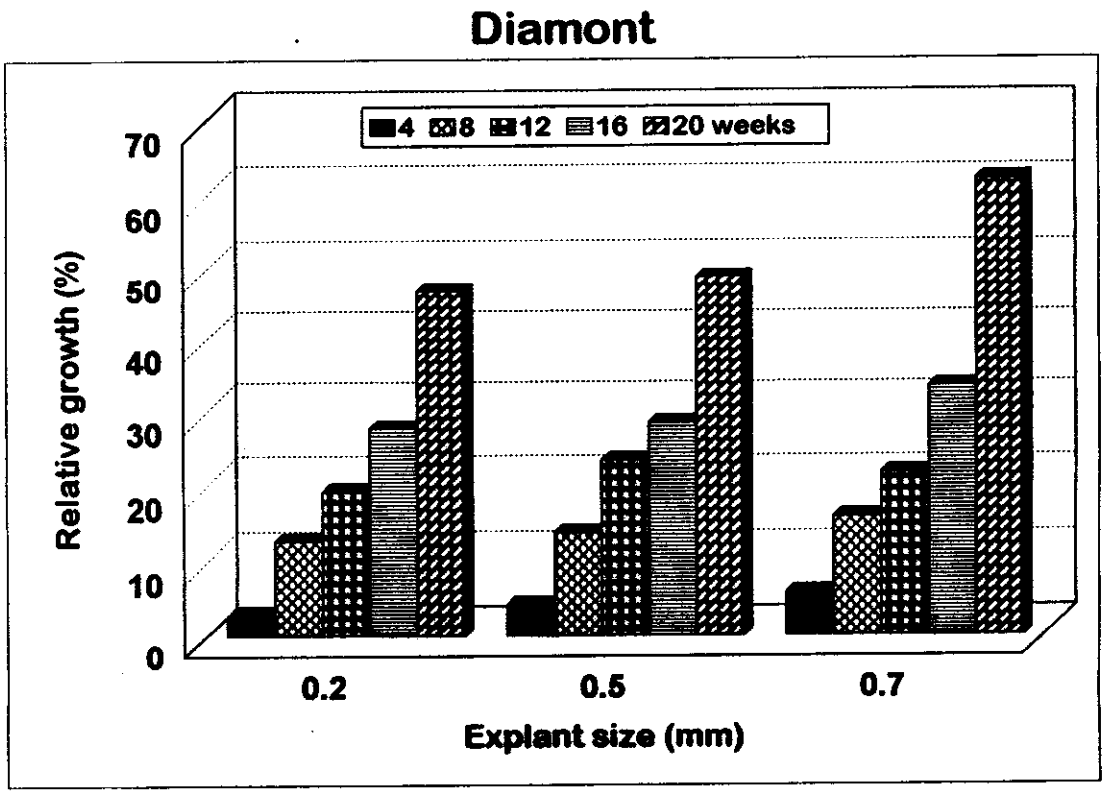


Fig. (11): Effect of different concentrations of Salicylic acid and size of explant on *in vitro* relative growth of potato cvs. Spunta and Diamont.

Fig. (12): Effect of Salicylic acid (Sal.A.) concentrations (ppm) on growth rate of potato plantlets (Spunta cv.) after 1-5 months *in vitro*.

5-3. -Effect of salicylic acid on elimination of potato viruses:

Effect of different concentrations of salicylic acid on virus elimination from the *in vitro* cultured meristems was present in **Tables (19 & 20).**

Data in **Table (19)** showed that, the different concentrations of Salicylic acid had no clear effect on elimination of viruses from plantlets of Spunta cv. particularly those developed from meristem tips with 0.2 mm long compared with control treatment. In control treatment 2/5 of the developed plantlets were virus-free, while only 1/5 was virus-free. Of 20 plantlets tested for all Salicylic acid concentrations 2, 7 and 8 plantlets were found to be infected with PVX, PVY and PVX + PVY, respectively.

Table (19): Effect of different concentration of salicylic acid on elimination of potato viruses using different size of explant in Spunta cultivar.

Size of explant (mm)	Salicylic acid conc. (ppm)	Detected viruses*							Virus-free
		PVX	PVY	PLRV	PVX + PVY	PVX + PLRV	PVY + PLRV	PVX + PVY + PLRV	
0.2	0	-	3	-	-	-	-	-	2
	10	1	1	-	2	-	-	-	1
	20	1	2	-	2	-	-	-	-
	40	-	1	-	4	-	-	-	-
Total		2	7	-	8	-	-	-	3
0.5	0	-	1	-	-	-	3	-	1
	10	1	1	-	1	-	-	-	2
	20	2	1	-	2	-	-	-	-
	40	-	-	-	5	-	-	-	-
Total		3	3	-	8	-	3	-	3
0.7	0	1	1	-	2	-	1	-	-
	10	-	3	-	-	-	-	-	2
	20	1	2	-	2	-	-	-	-
	40	-	3	-	1	-	-	-	1
Total		2	9	-	5	-	1	-	3
Grand Total		7	19	-	21	-	4	-	12

* The ELISA test was carried out on 5 plantlets per each treatment.

In case of the medium size of explant i.e. 0.5 mm long, the Salicylic acid at 10 ppm produced 2/5 virus-free plantlets compared with only 1/5 in case of control treatment (without Salicylic acid). Of 20 plantlets tested for that length, the majority (8/20) was still infected with PVX + PVY, while 3/20 were still infected with PVX, PVY or PVY + PLRV. The highest infection with PVY (3/5) and PVX (2/5) were associated with control treatment and 20 ppm concentration of Salicylic acid, respectively. As for the larger size of explant i.e. 0.7 mm long, the same data proved highly efficiency of Salicylic acid particularly at concentrations of 10 and 40 ppm. At these concentrations 2 out 5 and 1 out 5 tested plantlets, in respective were virus-free compared with 0/5 in case of control treatment. It could be noticed also that the majority of tested plantlets developed from the larger size of explant were still infected with PVY (9/20) followed by those infected with PVX + PVY (5/20), PVX (2/20) and PVY + PLRV (1/20).

Regarding Diamont cultivar **(Table, 20),** the salicylic acid treatment seems to be more effective in elimination of potato viruses from its cultured explants if compared with Spunta cv. Of total 20 plantlets tested (developed from 0.7 mm long explants) 8 plantlets were virus-free. Of those virus-free plantlets 3/5, 2/5 and 3/5 were produced at concentrations 10, 20 and 40 ppm of Salicylic acid, respectively. At 20 ppm of Salicylic acid, 100 % of ELISA tested plantlets (5/5) were virus-free compared with only 80 % (4/5) of plantlets developed in control and 10 ppm Salicylic acid treatments while only 20 % (1/5) of those developed at 40 ppm were virus-free.

Table (20): Effect of different concentration of salicylic acid on elimination of potato viruses using different size of explant of Diamont cultivar.

Size of explant (mm)	Salicylic acid conc. (ppm)	Detected viruses*							Virus-free
		PVX	PVY	PLRV	PVX + PVY	PVX + PLRV	PVY + PLRV	PVX + PVY + PLRV	
0.2	0	-	-	1	-	-	1	-	3
	10	3	-	-	-	1	-	-	1
	20	2	-	-	-	-	-	1	2
	40	1	1	-	-	-	-	-	3
Total		6	1	1	-	1	1	1	9
0.5	0	-	1	-	-	-	-	-	4
	10	-	1	-	-	-	-	-	4
	20	-	-	-	-	-	-	-	5
	40	1	-	-	-	2	-	-	1
Total		1	2	-	-	2	-	-	14
0.7	0	-	5	-	-	-	-	-	-
	10	1	-	-	-	-	1	-	3
	20	1	-	-	-	1	1	-	2
	40	-	2	-	-	-	-	-	3
Total		2	7	-	-	1	2	-	8
Grand Total		9	11	1	-	4	3	1	31

* The ELISA test was carried out on 5 plantlets per each treatment.

5-4. -Effect of salicylic acid on the *in vitro* potato microtubers production:

Data about effect of different concentration on microtubers production for both virus-free plantlets of potato cvs. Spunta and Diamont as well as the plantlets infected with PVX, PVY and PLRV either singly or in combination were tabulated in **Tables (21 & 22).**

As for *in vitro* tuberization of potato cv. Spunta , the data in **Table (21)** and **Fig. (13)** showed that, at 10 or 40 ppm of salicylic acid, total number, total weight and volume of microtubers per plantlet was significantly higher in virus-free plantlets than in plants infected with PVX, PVY either singly or

Table (21): *Effect of explant size and different concentrations of Salicylic acid on the in vitro microtuber production of plantlets of potato cvs. Spunta virus-free or infected with PVX, PVY and/or PLRV singly or in combination.*

Virus-Status	Total No. of microtuber per plantlet @				Total Wt. of microtuber in g per plantlet				Average Wt. of a single microtuber in mg				Total Volume in ml. of microtuber per plantlet				Average Volume in ml.of a single microtuber			
	Control	10 ppm	20 ppm	40 ppm	Control	10 ppm	20 ppm	40 ppm	Control	10 ppm	20 ppm	40 ppm	Control	10 ppm	20 ppm	40 ppm	Control	10 ppm	20 ppm	40 ppm
PVX	14.8	10.0	9.30	--	3.64	2.84	3.16	--	245.9	284.0	339.8	--	3.50	2.13	2.75	--	0.24	0.28	0.30	--
PVY	8.0	11.8	11.8	8.8	2.91	2.83	2.87	2.31	363.8	239.8	243.2	262.5	2.50	2.63	2.25	1.80	0.31	0.22	0.19	0.20
PLRV	-- [A]	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
PVX + PVY	14.8	9.0	8.3	9.8	2.97	1.56	2.32	1.46	200.7	173.3	279.5	149.0	3.00	1.53	2.63	1.35	0.20	0.17	0.32	0.14
PVX + PLRV	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
PVY + PLRV	8.5	--	--	--	2.36	--	--	--	277.6	--	--	--	2.18	--	--	--	0.26	--	--	--
PVX + PVY + PLRV	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Virus-free	16.0	17.5	--	16.5	3.98	3.26	--	4.19	248.9	186.3	--	253.9	6.38	2.60	--	4.13	0.40	0.15	--	0.25
L.S.D., at 0.05	1.00	2.27	1.63	N.S.	0.32	0.03	N.S.	N.S.	29.1	26.9	32.5	N.S.	0.80	1.42	N.S.	1.42	0.03	0.03	0.03	N.S.

@ = A single plantlet divided into five segments and cultured together in one jar.
A = Cases of virus status not detected by ELISA test.

Table (22): *Effect of explant size and different concentrations of Salicylic acid on the in vitro microtuber production of plantlets of potato cvs. Diamont virus-free or infected with PVX, PVY and/or PLRV singly or in combination.*

Virus-Status	Total No. of microtuber per plantlet @				Total Wt. of microtuber in g per plantlet				Average Wt. of a single microtuber in mg				Total Volume in ml. of microtuber per plantlet				Average Volume in ml.of a single microtuber			
	Control	10 ppm	20 ppm	40 ppm	Control	10 ppm	20 ppm	40 ppm	Control	10 ppm	20 ppm	40 ppm	Control	10 ppm	20 ppm	40 ppm	Control	10 ppm	20 ppm	40 ppm
PVX	– A	7.8	12.3	14.3	–	1.06	1.72	1.73	–	135.9	139.8	121.0	–	0.87	1.75	1.62	–	0.11	0.14	0.11
PVY	8.0	–	–	17.0	1.88	–	–	2.09	235.0	–	–	122.9	1.95	–	–	2.42	0.24	–	–	0.14
PLRV	6.3	–	–	–	2.21	–	–	–	350.8	–	–	–	2.50	–	–	–	0.40	–	–	–
PVX + PVY	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PVX + PLRV	–	12.0	9.5	–	–	1.57	1.46	–	–	130.8	153.7	–	–	1.70	2.20	–	–	0.14	0.23	–
PVY + PLRV	5.6	9.3	9.3	–	2.05	1.15	1.38	–	366.1	123.7	148.4	–	1.47	1.75	1.70	–	0.26	0.19	0.18	–
PVX + PVY + PLRV	–	–	11.5	–	–	–	1.57	–	–	–	136.5	–	–	–	1.75	–	–	–	0.15	–
Virus-free	9.0	9.5	9.8	10.8	2.65	2.61	2.67	2.03	294.4	274.7	272.4	188.0	2.02	2.20	2.58	1.59	0.22	0.24	0.26	0.15
L.S.D., at 0.05	2.33	2.25	2.26	3.01	N.S.	0.86	0.62	N.S.	N.S.	113.0	N.S.	44.0	N.S.	0.66	0.44	0.62	N.S	0.08	0.07	0.04

@ = *A single plantlet divided into five segments and cultured together in one jar.*
A = Cases of virus status not detected by ELISA test.

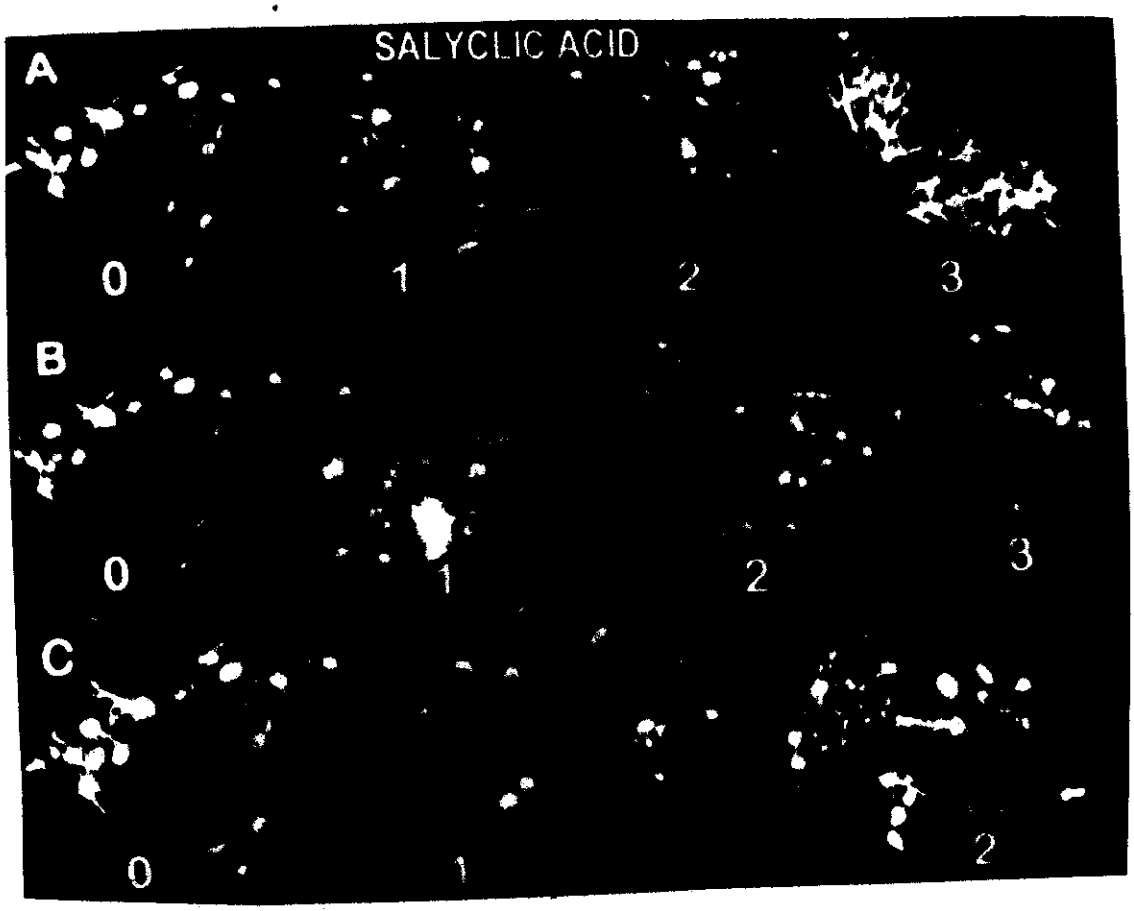


Fig. (13): Effect of Salicylic acid at 10 ppm (A), 20 ppm (B), and 40 ppm (C) on microtubers production of virus-free plantlets (0) as well as those still infected with: PVX(1-A,B), PVY(2-A,B, 1-C), PVY+PVX(3-A,B, 2-C). (all plantlets developed from 0.5 mm long explant of Spunta cv.).

combination. Compared with virus-free plantlets at concentration of 10 ppm, reduction in number of microtuber per plantlet was 42.9, 32.6 and 48.6 %, in total weight of microtubers per plantlets was 12.9, 13.2, and 52.1 %, respectively due to infection with PVX, PVY or PVX + PVY, respectively. Due to infection with PVY alone or combined with PVX in treatment of 40 ppm, number of microtubers per plantlets was reduced by 46.7 and 40.6 % and total weight of microtubers was reduced by 44.9 and 65.2 %, respectively. At concentration of 40 ppm , infection with PVX + PVY only reduced average weight of individual microtuber by 41.3 compared with virus-free case. At 20 ppm concentration, no virus-free plantlets of potato cv. Spunta were detected.

Respecting with potato cv. Diamont, the data in **Table (22)** stated that, at different concentrations of Salicylic acid, the total number of microtubers per plantlets was significantly equal or higher in plantlets infected with different tested potato viruses compared with virus-free plantlets. This was in contrast with total weight of microtubers per plantlet, which was significantly higher in virus-free plantlets compared with those infected with different viruses. At concentration of 10 ppm of Salicylic acid, total weight of microtubers per plantlets was reduced by 59.4, 39.8 and 55.9 % due to infection with PVX, PVX + PLRV and PVY + PLRV, respectively. The latter three infections at same concentration of Salicylic acid reduced average weight of single microtuber by 50.5, 52.4 and 55.0 %, respectively, however PVX-infection only reduced total volume of microtubers per plantlet by 60.6 % compared with virus-free plantlets. Infection with PVX alone or combined with PLRV reduced average volume of single microtuber by 54.2 and 41.7 %, respectively.

With regard to concentration of 20 ppm of Salicylic acid, infections with PVX, PVX + PLRV, PVY + PLRV, PVX + PVY + PLRV reduced total weight of microtubers per plantlet by 35.6, 45.3, 48.3 and 41.2 %, respectively. The total

volume of microtubers per plantlet was reduced due these infection by 32.2, 14.7, 34.1 and 32.2 %, and average volume of a single microtuber by 46.2, 38.5, 26.9, and 42.3 %, respectively. At concentration of 40 ppm, detected infections i.e. PVX and PVY each alone had no significant effects on total weight of microtubers per plantlets but they reduced average weight of single microtuber by 35.6 and 34.6 %, respectively.

6 -Effect of combination between gamma irradiation of infected potato tubers and size of explant:

In this experiment, potato tubers of Spunta and Diamont cvs., collected from potato plants infected with PVX, PVY and PLRV were sprouted and exposed to gamma irradiation at doses of 0, 15, 30 or 45 Gray. Explants were excised (from sprouts of irradiated potato tubers) at lengths of 0.2, 0.5 and 0.7 mm and transferred to MS-culture medium. For each potato cultivar, given size and dose of irradiation 24 tips were used. Through out 20 weeks of incubation, the survival percentage, the *in vitro* relative growth of potato plantlets and detection of status of viruses (PVX, PVY and PLRV) as affected by combination between irradiation and size of explant were accomplished as described before.

Table (23): Effect of irradiation and size of explant on percentage of survived plantlets for potato cultivars Spunta and Diamont.

Cultivar	Irradiation dose (Gray)	Different size of explant (mm)					
		0.2		0.5		0.7	
		Number	%	Number	%	Number	%
Spunta	0	17	70.8	17	70.8	20	83.3
	15	16	66.7	19	79.2	19	79.2
	30	8	33.3	10	41.7	11	45.8
	45	8	33.3	9	37.5	9	37.5
Diamont	0	14	58.3	16	66.7	20	83.3
	15	8	33.3	14	58.3	14	58.3
	30	7	29.2	14	58.3	14	58.3
	45	6	25.0	10	41.7	10	41.7

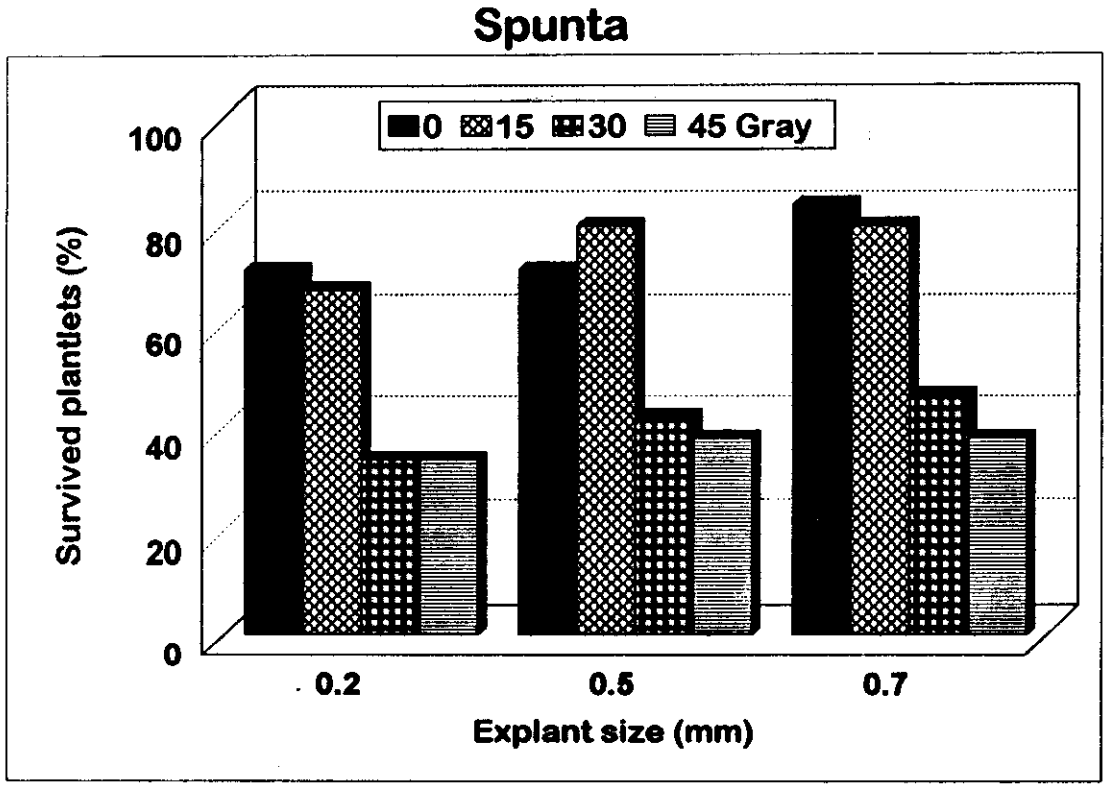
6-1. -Effect of irradiation on survival percentage:

The data presented in **Table (23) and Fig. (14)** indicated that percentages of survived plantlets of both potato cvs. Spunta and Diamont were affected negatively by irradiation treatment. The dangerous effect of gamma irradiation on survivals, in general, was slightly decreased with increasing length of the excised explants. The negative effect was more conspicuous at doses of 30 and 45 Gray than at 15 Gray. After 20 weeks from incubation, percentages of survived plantlets were 83.3, 79.2, 45.8 and 37.5% for potato cv. Spunta and 83.3, 58.3, 58.3 and 41.7% for potato cv. Diamont at irradiation doses of 0, 15, 30 and 45 Gray, respectively.

6-2. -Effect of irradiation on rate of growth:

Results in **Tables (24 & 25)** and **Figs. (15 & 16)** revealed that the *in vitro* growth rate of potato plantlets of both potato cultivars Spunta and Diamont was clearly increased with increasing length of explant from 0.2 mm to 0.7 mm as well as by prolongation of incubation period up to 20 weeks.

The obtained data showed also that, the *in vitro* relative growth developed from different size of explants of the tested potato cultivars Spunta and Diamont might be responded differently to gamma irradiation during incubation periods. In case of Spunta cv. **(Table, 24)**, the growth rate of explants excised at length of 0.2 mm from non irradiated potato tubers was better than growth rate of explants with same length and taken from irradiated potato tubers. In contrast, the highest growth rate in case of explants taken at length of 0.5 or 0.7 mm was associated with those excised from potato tuber previously exposed to irradiation at dose of 30 Gray compared with those excised from control tubers (non-irradiated). In contrast with Spunta cv., all tested doses of gamma



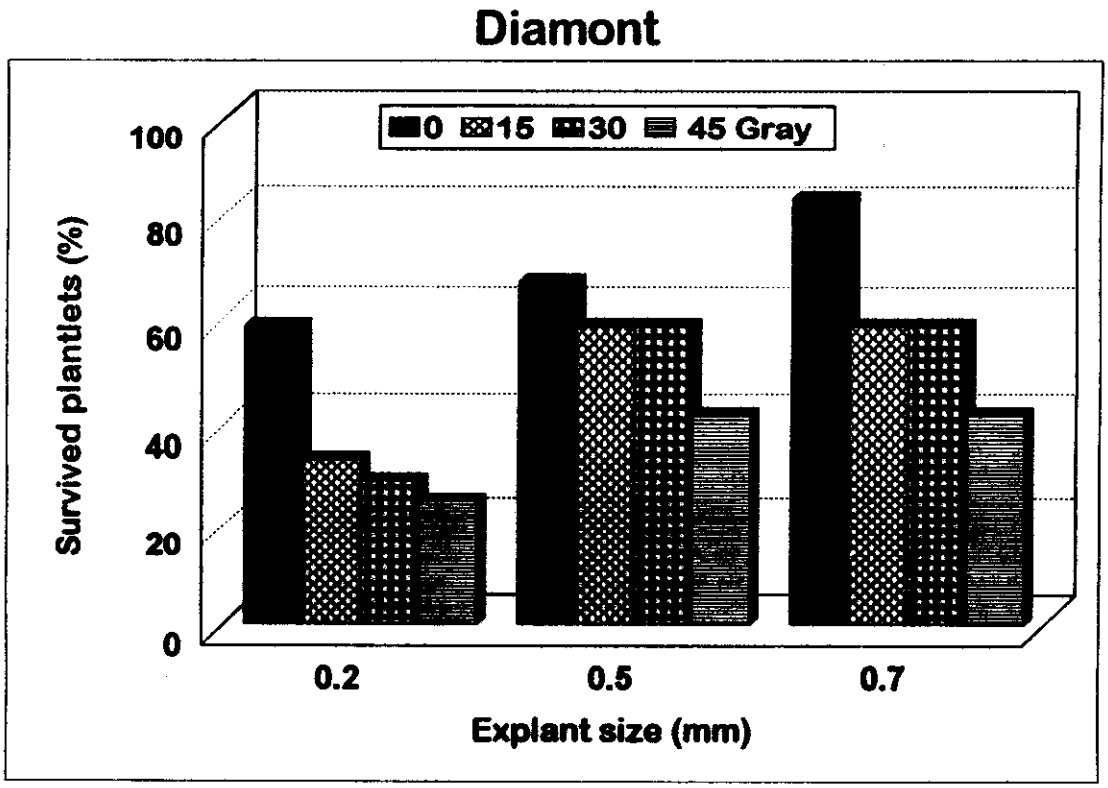


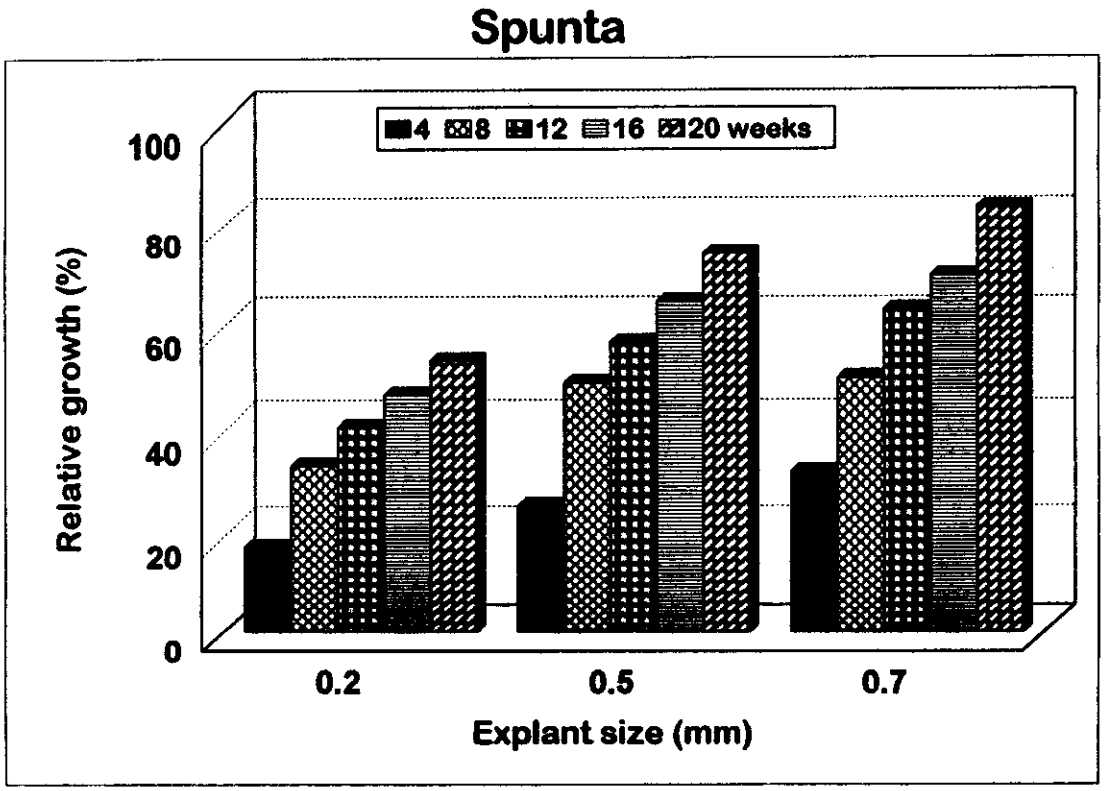
Fig. (14): Effect of pretreatment with different doses of gamma irradiation and size of explant on survival percentages of potato cvs. Spunta and Diamont.

Table (24): Effect of irradiation on the in vitro growth rate of potato cv. Spunta through out 20 weeks of incubation.

Size of explant (mm)	Irradiation dose (Gray)	Growth Rate (%)				
		4 weeks	8 weeks	12 weeks	16 weeks	20 weeks
0.2	0	14.17	35.00	39.17	52.50	62.50
	15	22.50	26.67	40.83	47.50	52.50
	30	18.26	32.38	41.82	45.45	53.33
	45	16.52	39.05	40.70	40.90	44.71
Mean		17.86	33.28	40.56	46.59	53.26
0.5	0	16.67	47.50	52.50	65.80	79.20
	15	33.91	61.76	68.69	71.30	75.65
	30	32.50	48.00	62.33	68.00	83.33
	45	20.00	38.26	43.48	53.68	58.57
Mean		25.77	48.88	56.75	64.70	74.19
0.7	0	19.17	39.10	62.72	77.27	89.10
	15	38.26	55.65	70.43	76.52	81.73
	30	45.83	62.86	68.57	69.52	96.36
	45	25.45	41.82	50.91	56.00	67.69
Mean		32.18	49.86	63.16	69.83	83.72

Table (25): Effect of irradiation on the growth and development (growth rate) of potato cv. Diamont cultured *in vitro* through 20 weeks.

Size of explant (mm)	Irradiation dose (Gray)	Growth Rate (%) after				
		4 weeks	8 weeks	12 weeks	16 weeks	20 weeks
0.2	0	5.45	29.09	40.00	45.45	63.64
	15	0.00	6.00	8.00	22.00	40.00
	30	2.00	8.00	18.00	20.00	48.00
	45	2.00	6.00	14.00	20.00	30.00
Mean		2.36	12.27	20.00	26.86	45.41
0.5	0	8.33	33.33	40.00	48.33	65.00
	15	1.80	4.00	12.00	22.00	48.00
	30	3.00	10.90	18.18	34.55	56.36
	45	1.70	6.70	16.70	20.00	40.00
Mean		3.71	13.73	21.72	31.22	52.34
0.7	0	11.60	20.00	28.33	48.33	83.33
	15	3.30	6.70	21.70	28.30	53.30
	30	3.33	11.66	18.33	30.00	55.00
	45	1.70	6.70	15.00	28.30	40.00
Mean		4.98	11.27	20.84	33.73	57.91



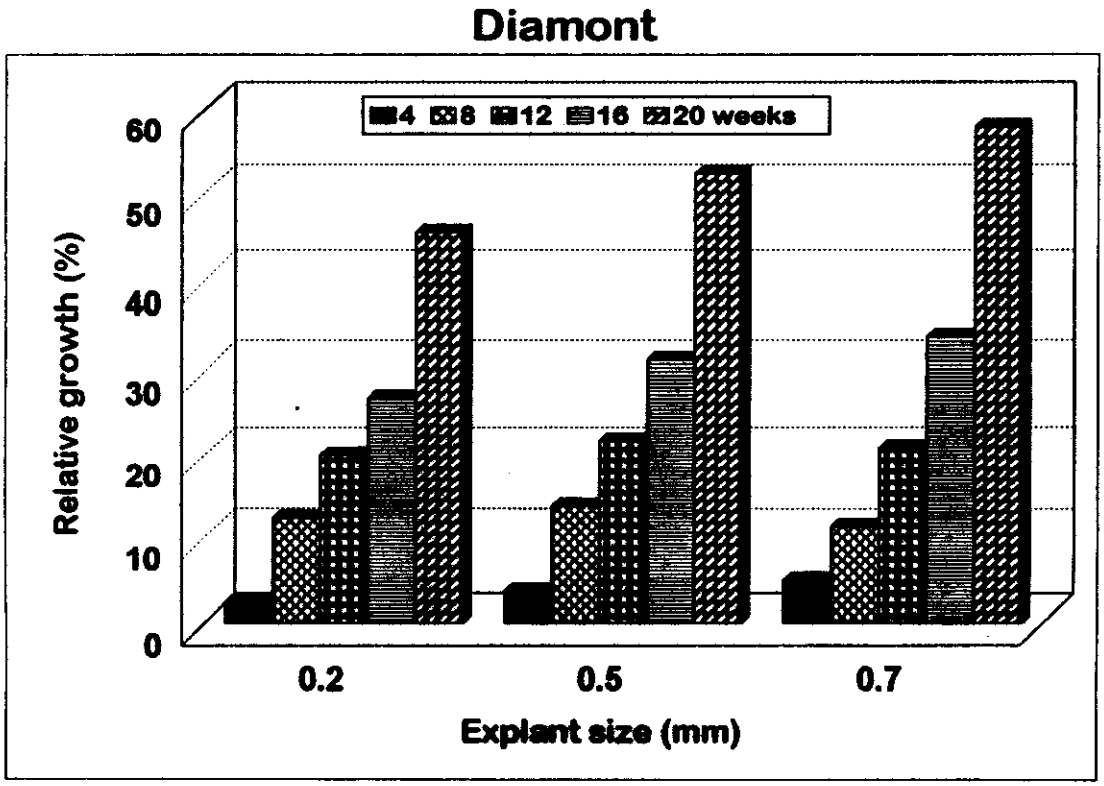


Fig. (15): Effect of pretreatment with different doses of gamma irradiation and size of explant on *in vitro* growth rate of potato cvs. Spunta and Diamont.

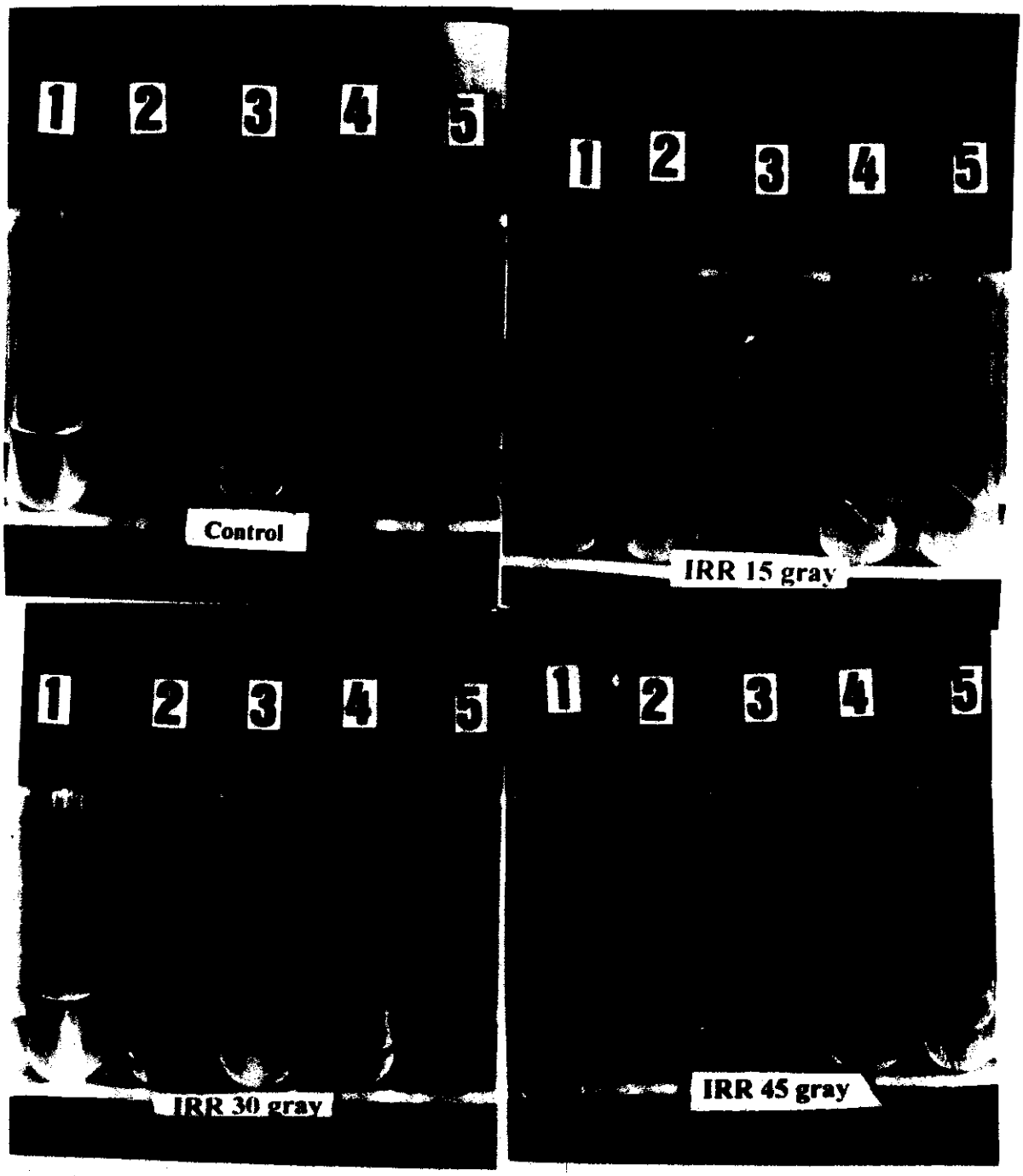


Fig. (16): Effect of pretreatment with different doses of gamma irradiation on growth of potato plantlets (Spunta cv.), after 1-5 months *in vitro*.

-irradiation seemed to be harmful to the *in vitro* growth rate of Diamont cv. **(Table, 25)**.

From aforementioned data, it could be concluded that gamma irradiation at dose of 30 Gray has some promoting effect on growth rate of 0.5 and 0.7 mm explants of Spunta but not Diamont potato cvs.

6-3. -Effect of radiation on elimination of potato viruses :

Effect of different doses of irradiation on elimination of potato viruses PVX, PVY and PLRV in Spunta and Diamont cultivars was demonstrated in **Tables (26 & 27).** The tabulated data presented that irradiation treatments were more beneficial for production of virus-free plantlets especially from potato cv. Diamont **(Table, 27)**. In this regard, out of total 60 plantlets subjected to ELISA test (for different doses of irradiation and size of explant), 30 (50%) plantlets of Diamont cv. were virus-free compares with only 5 plantlets of Spunta cv. **(Table, 26)**. The numbers of the virus-free plantlets of Diamont cv., produced from 0.2, 0.5, and 0.7 mm explants were 11/20, 15/20 and 4/20, respectively. The highest percentage of virus-freedom (100 %) was associated with plantlets developed from 0.5 mm explants excised from sprouted tubers exposed to gamma irradiation at 30 Gray. However, exposing sprouted tubers of Spunta cv. to irradiation doses 15 and 30 Gray resulted in only 1/5 (20 %) virus-free plantlet for each dose. It could be noticed, in general, that the number of virus-free plantlets obtained by irradiation treatment in Diamont was higher than Spunta cultivar which reflect the genotype effect.

As for effect of irradiation on elimination of specific virus infection, the same data proved that the infections with PVY either singly or combined with PLRV in case of Spunta cv. were more difficult to eradication by irradiation than

Table (26): Effect of different doses of gamma irradiation on elimination of potato viruses infected Spunta cultivar.

Size of explant (mm)	Dose of Irradiation (Gray.)	Detected viruses*							Virus-free
		PVX	PVY	PLRV	PVX + PVY	PVX + PLRV	PVY + PLRV	PVX + PVY + PLRV	
0.2	0	-	3	-	-	-	-	-	2
	15	1	4	-	-	-	-	-	-
	30	-	1	-	1	-	2	1	-
	45	-	1	-	1	-	3	-	-
Total		1	9	-	2	-	5	1	2
0.5	0	-	1	-	-	-	3	-	1
	15	-	-	-	-	-	3	1	1
	30	-	4	-	-	-	-	1	-
	45	-	-	-	4	-	-	-	1
Total		-	5	-	4	-	6	2	3
0.7	0	1	1	-	2	-	1	-	-
	15	-	5	-	-	-	-	-	-
	30	-	3	-	1	-	-	1	-
	45	-	1	1	-	-	3	-	-
Total		1	10	1	3	-	4	1	-
Grand Total		2	24	1	9	-	15	4	5

* The ELISA test was carried out on 5 plantlets per each treatment.

Table (27): Effect of different doses of gamma irradiation on elimination of potato viruses infected Diamont cultivar.

Size of explant (mm)	Dose of irradiation (Gray.)	Detected viruses*							Virus-free
		PVX	PVY	PLRV	PVX + PVY	PVX + PLRV	PVY + PLRV	PVX + PVY + PLRV	
0.2	0	-	-	1	-	-	1	-	3
	15	-	3	-	-	-	-	-	2
	30	-	2	1	-	-	-	-	2
	45	1	-	-	-	-	-	-	4
Total		1	5	2	-	-	1	-	11
0.5	0	-	1	-	-	-	-	-	4
	15	1	-	-	-	-	-	-	4
	30	-	-	-	-	-	-	-	5
	45	1	2	-	-	-	-	-	2
Total		2	3	-	-	-	-	-	15
0.7	0	-	5	-	-	-	-	-	-
	15	1	-	1	-	-	-	-	3
	30	-	2	2	-	-	-	-	1
	45	-	-	3	-	1	1	-	-
Total		1	7	6	-	1	1	-	4
Grand Total		4	15	8	-	1	3	-	30

* The ELISA test was carried out on 5 plantlets per each treatment.

to eradication by irradiation than other cases of virus-infections. These infections were detected in 40 % (24/60) and 25 % (15/60) of the tested plantlets, respectively **(Table 26)**. In potato cv. Diamont **(Table 27)**, the singular infections with PVY or PLRV were the most resistant to elimination by irradiation treatments. They were detected in 25 % (15/60) and 13.3 % (8/60) of tested plantlets, respectively. On the other hand, the combined infections with PVX + PVY and PVX + PVY + PLRV were completely absent from tested plantlets (0/60) of Diamont cv., but they were detected in 15 % (9/60) and 6.7 % (4/60), respectively from tested plantlets of Spunta cv.

6-4. –Effect of pretreatment with irradiation on in vitro microtuber production:

The data in **Table (28)** explored that the 0.5 mm long explants excised from sprouted potato tubers, exposed to gamma irradiation at 15 and 45 Gy but not 30 Gy of gamma irradiation, and cultured on MS-medium, resulted in production of virus-free plantlets. Microtuber productivity of plantlets, which were still infected (after irradiation) with different potato viruses, was significantly less than those were virus-free **(see Fig., 17 A, B & C)**. In case of plantlets of 15 Gy treatment but still infected with PVX, PVY, PVX + PLRV, or PVX + PVY + PLRV, the total weight of microtubers per plantlet, was reduced by 8.5, 44.6, 26.3, and 73.4 %, the average weight of single microtuber was reduced by 26.1, 43.6, 18.5, and 41.3 %, the total volume of microtubers per plantlet was reduced by 21.8, 56.3, 46.8, and 72.5 %, and the average volume of single microtuber was reduced by 36.8, 55.3, 42.1, and 39.5 %, respectively compared with virus-free plantlets

Table (28): *Effect of different doses of gamma irradiation on the in vitro microtuber production of plantlets of potato cvs. Spunta virus-free or infected with PVX, PVY and/or PLRV singly or in combination.*

Virus-Status	Total No. of microtuber per plantlet @				Total Wt. of microtuber in g per plantlet				Average Wt. of a single microtuber in mg				Total Volume in ml. of microtuber per plantlet				Average Volume in ml.of a single microtuber			
	Control	15 Gy	30 Gy	45 Gy	Control	15 Gy	30 Gy	45 Gy	Control	15 Gy	30 Gy	45 Gy	Control	15 Gy	30 Gy	45 Gy	Control	15 Gy	30 Gy	45 Gy
PVX	14.8	13.0	--	--	3.64	2.89	--	--	245.9	222.3	--	--	3.50	3.13	--	--	0.24	0.24	--	--
PVY	8.0	10.3	7.5	8.0	2.91	1.75	1.98	2.70	363.8	169.9	264.0	337.5	2.50	1.75	1.88	2.00	0.31	0.17	0.26	0.25
PLRV	-- [A]	--	--	13.3	--	--	--	3.49	--	--	--	262.4	--	--	--	2.50	--	--	--	0.19
PVX + PVY	14.8	--	8.0	13.0	2.97	--	1.75	1.46	200.7	--	218.8	112.3	3.00	--	1.63	1.50	0.20	--	0.20	0.12
PVX + PLRV	--	9.50	--	--	--	2.33	--	--	--	245.3	--	--	--	2.13	--	--	--	0.22	--	--
PVY + PLRV	8.5	--	13.3	13.5	2.36	--	2.53	2.58	277.6	--	190.2	191.0	2.18	--	2.20	2.40	0.26	--	0.17	0.18
PVX + PVY + PLRV	--	4.75	8.5	--	--	0.84	1.53	--	--	176.8	180.0	--	--	1.10	1.70	--	--	0.23	0.20	--
Virus-free	16.0	10.5	--	11.8	3.98	3.16	--	4.12	248.9	301.0	--	364.6	6.38	4.00	--	6.25	0.40	0.38	--	0.53
L.S.D., at 0.05	0.97	0.78	0.43	0.27	0.32	0.62	0.04	0.17	29.1	6.9	10.7	16.8	0.80	0.54	0.19	0.21	0.03	0.01	0.03	0.03

@ = A single plantlet divided into five segments and cultured together in one jar.
A = Cases of virus status not detected by ELISA test.

Table (29): *Effect different doses of gamma irradiation on the in vitro microtuber production of plantlets of potato cvs. Diamont virus-free or infected with PVX, PVY and/or PLRV singly or in combination.*

Virus-Status	Total No. of microtuber per plantlet @				Total Wt. of microtuber in g per plantlet				Average Wt. of a single microtuber in mg				Total Volume in ml. of microtuber per plantlet				Average Volume in ml.of a single microtuber			
	Control	15 Gy	30 Gy	45 Gy	Control	15 Gy	30 Gy	45 Gy	Control	15 Gy	30 Gy	45 Gy	Control	15 Gy	30 Gy	45 Gy	Control	15 Gy	30 Gy	45 Gy
PVX	-- [A]	6.8	--	10.0	--	2.05	--	1.98	--	301.5	--	198.0	--	2.97	--	2.00	--	0.44	--	0.20
PVY	8.0	8.8	11.5	--	1.88	1.63	1.42	--	235.0	185.2	123.5	--	1.95	2.17	2.10	--	0.24	0.25	0.18	--
PLRV	6.3	8.5	12.5	15.8	2.21	1.94	2.06	2.91	350.8	228.2	164.8	184.2	2.50	1.37	1.39	2.15	0.40	0.16	0.11	0.14
PVX + PVY	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
PVX + PLRV	--	--	--	15.8	--	--	--	2.27	--	--	--	143.7	--	--	--	1.72	--	--	--	0.11
PVY + PLRV	5.6	--	--	11.0	2.05	--	--	1.71	366.1	--	--	155.5	1.47	--	--	1.45	0.26	--	--	0.13
PVX + PVY + PLRV	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Virus-free	9.0	11.8	10.0	10.5	2.65	2.52	2.34	1.77	294.4	213.6	234.0	168.6	2.02	1.30	1.55	3.17	0.22	0.11	0.16	0.30
L.S.D., at 0.05	2.33	2.72	1.73	3.48	N.S.	N.S.	N.S.	0.84	N.S.	N.S,	43.0	N.S.	N.S.	1.04	N.S.	0.58	N.S	0.08	N.S	0.05

@ = *A single plantlet divided into five segments and cultured together in one jar.*

A = Cases of virus status not detected by ELISA test.

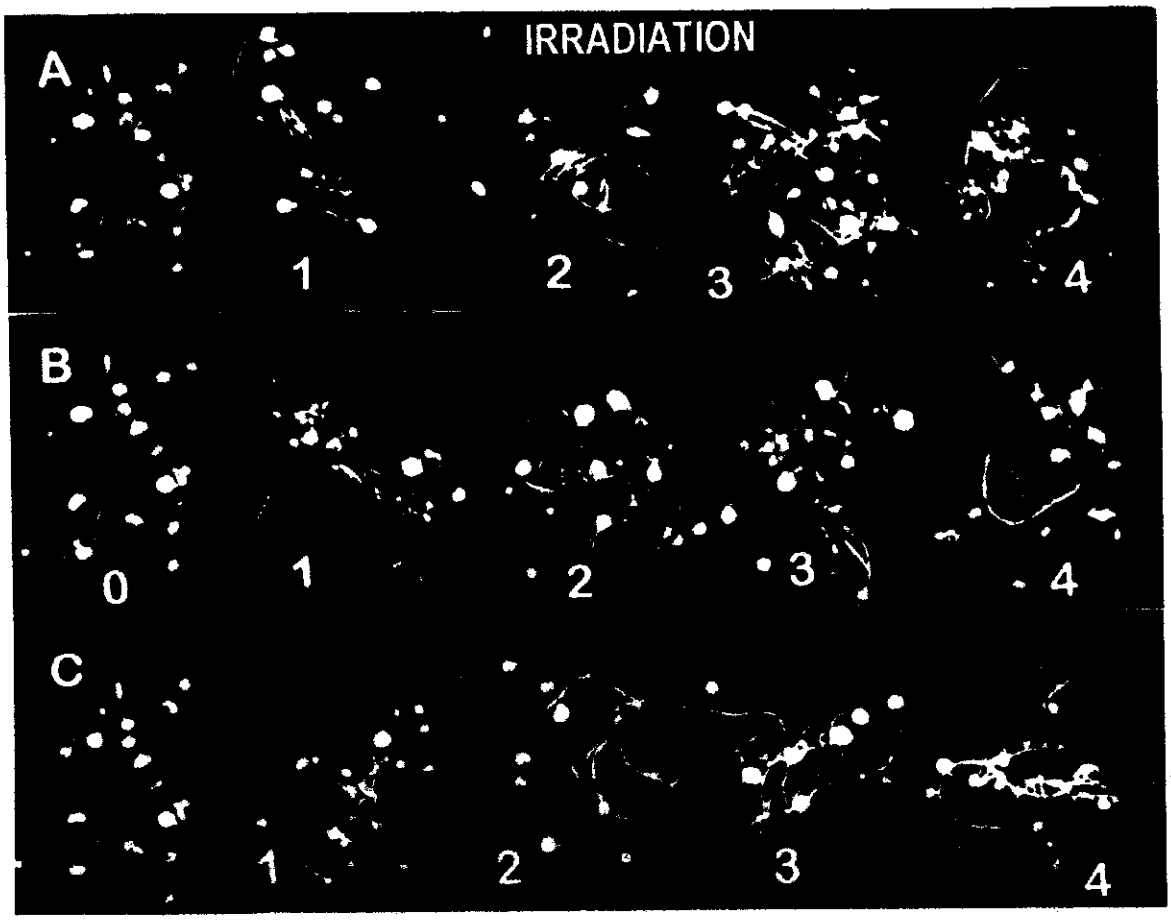


Fig. (17): Effect of pretreatment with 15 (A), 30 (B), and 30 (C) GY of gamma irradiation on microtubers production of virus-free plantlets (0) as well as those still infected with PVX (1-A), PVY (2-A, 1-B,C), PVX+PVY (2-B, 3-C), PLRV (2-C), PVY+PLRV (3-A, 4-C), PVX+PVY+PLRV (4-A,B) (all plantlets developed from 0.5 mm long explant of Spunta cv.).

As for plantlets developed from pretreatment with gamma irradiation at 45 Gy and still infected with PVY, PLRV, PVX + PVY or PVY + OLRV, the total weight of microtubers per plantlet was reduced by 34.5, 15.3, 64.6, and 37.4 %, the average weight of single microtuber was reduced by 7.4, 28.0, 69.2, and 47.6 %, the total volume of microtubers per plantlet was reduced by 68.0, 60.0, 76.0 and 61.6 %, and the average volume of single microtuber was reduced by 52.8, 64.2, 77.4 and 66.0 %, respectively.

It could be noticed also that, the combined infection PVX + PVY + PLRV (in case of 15 Gy treatment) or with PVY alone (in case of 45 Gy treatment) reduced the total number of microtubers per plantlet by 54.8 and 32.2 %, in respective, compared with healthy virus-free plantlets.

Respecting. with potato cv Diamont, the data in **Table (29)** viewed unexpected results. In case of irradiation at 15 Gy, the total number of microtubers per plantlets still infected with PVX, PVY, or PLRV, each alone, was reduced by 42.4, 25.4, and 28.0%, in respective compared with virus-free plantlet. At same dose of irradiation and same viral infections, both total weight of microtubers per plantlet and average volume of a single microtuber were not significantly differed, if compared with those of virus-free plantlets. However, both total volume of microtubers per plantlet and average volume of a single microtuber were significantly higher especially in plantlets infected with PVX alone if compared with those of virus-free plantlets. The latter trend was noticed also in case of 30 and 45 Gy irradiation treatments.

6-5. –Effect of irradiation on protein pattern:

Because exposing plants to gamma irradiation may affect their patterns for protein synthesis and metabolism, this experiment was conducted to investigate protein pattern in plantlets developed from irradiated potato tubers of Spunta and Diamont cultivars. Protein pattern as affected by different irradiation doses i.e. 15, 30 and 45 Gray was compared with that of healthy (virus-free) and

virus-infected plantlets developed from non-irradiated potato tubers. Protein extraction was obtained and separated by electrophoresis standard procedure. The obtained protein bands were stained with coomassie blue R 250.

Table (30): The effete of irradiation dosage on protein profile in the *in vitro* healthy and infected plantlets of Spunta and Diamont cultivars.

MW	Spunta					Diamont				
	Control		Irradiation			Control		Irradiation		
KDa	Heal. (1)	Inf. (2)	15 Gy	30 Gy	45 Gy	Heal. (1)	Inf. (2)	15 Gy	30 Gy	45 Gy
41	+	-	-	-	-	-	+	+	-	-
38	+	+	+	+	+	-	-	+	+	+
36	+	+	+	+	+	-	-	+	-	-
34	+	+	+	+	+	-	-	+	-	+
27	+	+	+	+	+	+	+	+	+	+
23	+	+	-	-	-	+	+	+	+	+
14	+	+	-	+	+	-	-	+	-	-
Total bands	7	6	4	5	5	2	3	7	3	4

Bands present = (+) Bands absent = (-)
(1) = Healthy , (2) = Infected.

The results obtained from SDS-PAGE analysis for potato cvs. Spunta and Diamont were shown in **Table (30)** and **Figs. (18 & 19)**. From these results it could be noticed that the healthy (virus-free) plantlets of Spunta cv. produced the highest number (7) of protein bands with molecular weight of 41, 38, 36, 34, 27, 23, and 14 Kda, respectively. It is clear also that the band with molecular weight of 41 Kda was completely disappeared from virus-infected (control) plantlets either non-irradiated or exposed to different doses of gamma irradiation. The band with molecular weight of 23 Kda was disappeared only from all plantlets of different doses of irradiation. However, the bands with 27, 34, 36, and 38 Kda seemed to be common bands for Spunta cv., and bands with 27 and 23 Kda

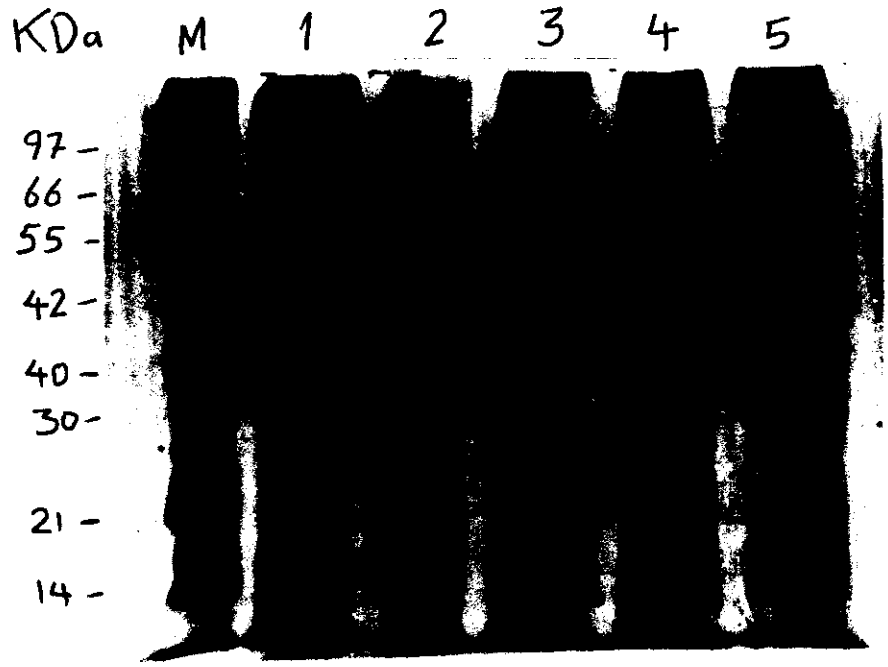


Fig. (18): SDS- PAGE for protein patterns in non-irradiated healthy and virus-infected plantlets of Spunta cultivar compared with those exposed to different doses of irradiation. (Molecular weight stand is indicated in KD at left side).

Lain M = protein Marker.
1 = Healthy Spunta cultivar.
2 = Infected Spunta cultivar.
3 = Irradiated plantlets at 15 GY.
4 = Irradiated plantlets at 30 GY.
5 = Irradiated plantlets at 45 GY.

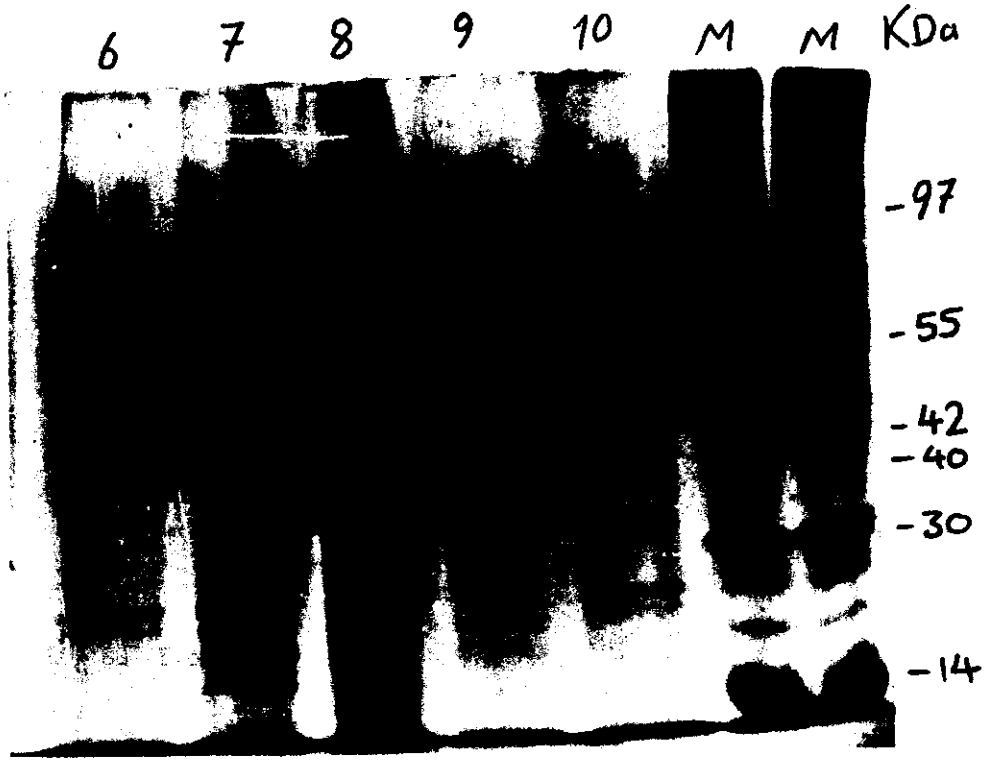


Fig. (19): SDS- PAGE for protein patterns in non-irradiated healthy and virus-infected plantlets of Diamont potato cv. compared with those exposed to different doses of irradiation.

Lain M = protein Marker.
Lain 6 = healthy Diamont cultivar.
7 = Infected Diamont cultivar.
8 = Irradiated plantlets at 15 GY.
9 = Irradiated plantlets at 30 GY.
10 = Irradiated plantlets at 45 GY.

only for Diamont cv. as they were detected in all healthy or virus-infected plantlets either, irradiated or non-irradiated. In Diamont cv., the SDS-PAGE analysis for the protein indicated that exposing to gamma irradiation at dose of 15 Gray produced the highest number of bands i.e. 7 bands. The band with molecular weight of 38 Kda seems to be common in all tested doses of gamma irradiation. In general, the bands with 41, 38 and 14 Kda were absent in plantlets exposed to 30 and 45 Gray compared with those of 15 Gray treatment. The band with 34 Kda was detected only in Diamont plantlets exposed to 15 or 45 Gray treatments. Finally, the number of detected protein bands were 2 and 3 for healthy and virus-infected plantlets of control treatments and 7, 3 and 4 for plantlets exposed to 15, 30 and 45 Gray, respectively.

From the above-mentioned results, Irradiation exhibited unbalance in the protein profile of potato cultivars used at this study. This behavior differs according to the dosage of radiation, the type of plant and the stage of viral infection.

7 –Minitubers productivity of different virus-free plants under greenhouse conditions as affected by different pre-treatments:

In this experiment the *in vitro* microtubers of virus-free plantlets of potato cvs. Spunta and Diamont resulted after exposing to different treatments i.e. Virazole or Salicylic acid or gamma irradiation were planted under greenhouse to evaluate their minitubers production and vegetative growth. Microtubers produced from virus-free plants developed from 0.5 mm long explants only were used in these studies. Five microtubers (replications) were used for each virus-free plantlet. It is interested to pointed that, no virus-free plants were obtained in both 20 ppm of Salicylic acid and 30 Gy of gamma irradiation treatments. The obtained results are tabulated in **Tables (31 and 32)**.

7-1. –Minitubers productivity of virus-free plants of Spunta cultivar:

As for a given character in Spunta potato cv., the data in **Table (31)** shows significant variations between different treatments. Statistical analysis of variance revealed that the virus-free plants developed after pretreatment with 40 ppm of Virazole produced the highest fresh weight of vegetative growth per plant (11.99 g) followed by those developed from control treatment (10.58 g) without significant differences between them. However, virus-free plants in treatments of 10 ppm of Salicylic acid (4.16 g/plant), Virazole (4.92 g) or 45 Gy irradiation (4.45 g) produced the lowest fresh weight of vegetative growth without significant differences in between. On the other hand, the highest significant increase in dry weight, compared with plants of control treatment, was produced by plants developed after 40 ppm of Virazole treatment (0.98 g/plant) and 40 ppm Salicylic acid treatment (0.81 g/plant), while plants developed after pre-irradiation with 15 Gy (0.28 g/plant) or 45 Gy (0.36 g/plant) gave the lowest values with clear significant differences in between

The same data showed also that, the average number of minitubers per plant produced by virus-free plants from all tested pre-treatments was significantly lower (2.25 – 4.5 minituber / plant) than that produced by virus-free plant from control treatment (7.5 minituber / plant). In this respect, the lowest numbers of minitubers were produced by pretreatment with 45 Gy of gamma irradiation (2.25 minituber/plant) 10 ppm of Virazole, 40 ppm of Salicylic acid (3 minituber/plant each) without significant variation in between.

Table (31): Minitubers productivity under greenhouse conditions using *in vitro* micotubers of virus-free plantlets of Spunta cv., developed from different treatments.

Treatment	Vegetative growth (in gm)		Total for minitubers/plant			Average of minituber	
	Fresh We.	Dry We.	Weight (gm)	Volume (ml)	Number	Weight (gm)	Volume (ml)
Control	10.58	0.63	26.77	15.00	7.50	3.57	2.00
Virazole at 10 ppm	4.92	0.62	30.09	30.38	3.00	10.03	10.13
Virazole at 20 ppm	8.61	0.64	14.74	13.78	3.50	4.21	3.93
Virazole at 40 ppm	11.99	0.98	29.86	25.25	4.00	7.47	6.31
Salicylic acid at 10 ppm	4.16	0.66	16.51	15.38	4.25	3.88	3.62
Salicylic acid at 40 ppm	6.82	0.81	20.63	19.13	3.00	6.88	6.38
Irradiation at 15 Gy	7.93	0.28	35.92	25.50	4.50	7.98	5.67
Irradiation at 45 Gy	4.45	0.36	18.43	16.00	2.25	8.19	7.11
L.S.D. at 0.05	1.71	0.01	0.45	1.29	0.73	1.39	1.44

As for total fresh weight of minitubers per virus-free plant, the same data proved that, the highest significant increases were produced by the virus-free plants developed from treatments of 15 Gy irradiation (35.92 g/plant), 10 ppm Virazole (30.09 g/plant), and 40 ppm Virazole (29.86 g/plant) compared with those of control treatment (26.77 g/plant). All other tested treatments affected fresh weight of minitubers of virus-free plants negatively and significantly compared with control treatment . In this regard, the greatest decreases however, were associated with virus-free plants developed after 10 ppm of Virazole (14.74 g), 10 ppm of Salicylic acid (16.51 g), and 45 Gy irradiation treatment (18.43 g) but with clear significant differences between them.

Respecting with total volume of minitubers produced by a virus-free plant, it is clear that, treatment with Virazole at 10 ppm concentration produces the highest significant increase in this respect (30.38 ml) followed by treatments of 15 Gy irradiation (25.50 ml), 40 ppm of Virazole (25.25 ml) without significant differences between the last two treatments and 40 ppm of Salicylic acid (19.13 ml). However, the lowest values of total volume of minituber/plant were given by virus-free plants of 20 ppm of Virazole (13.78 mi) and control treatment (15.0 ml) without significant variation in between.

It is worthy to state that, the average size of an individual minituber was significantly higher in all tested virus-free plants (5.67-10.13 ml/minituber) than that of control treatment (2.0 ml/minituber). The highest values in this regard, were associated with treatments of 10 ppm of Virazole (10.13 ml) and 45 Gy irradiation (7.11 ml). It could be noticed also that, the average weight of the individual minitubers produced by treatment of 10 ppm of Virazole (10.03 g), 15 Gy irradiation ((7.98 g), 40 ppm of Virazole (7.47 g), and 40 ppm of Salicylic acid (6.88 g) was significantly higher than that of control treatment ((3.57 g). The average weight of an individual minituber of the later treatment (control) was not

significantly varied with its corresponding values in treatments of 20 ppm of Virazole (4.21 g) and 10 ppm of Salicylic acid (3.88 g).

7-2. –Minitubers productivity of virus-free plants of Diamont cultivar:

In case of potato cv. Diamont, the data in **Table (32)** declared that, the highest significant increases in weight of vegetative growth for a virus-free plant was associated with treatments of 45 Gy irradiation (8.66 g) and 40 ppm of Virazole (8.33 g) followed by 40 ppm of Salicylic acid (7.05 g), while no significant differences were detected between control treatment (5.33 g) and treatments of 15 Gy irradiation (5.61 g), 30 Gy irradiation (6.64 g), 10 ppm of Virazole (6.42) and 20 ppm of Virazole (5.64 g). On the other hand, dry weight in treatments of 20 and 40 ppm of Salicylic acid (0.56-0.58 g) and 15 Gy irradiation (0.59 g) only was significant lower than in control treatment (0.63 g).

Virus-free plants produced through all tested treatments included control showed no significant variations in number of minitubers per plant (4.00-5.75), average weight of a single minituber (5.04-7.73 g) and volume of a single minituber (4.54-6.74 ml).

In Diamont cv., no one of different tested treatments could be able to increase total fresh weight of minituber per plant compared with control treatment. However, total fresh weight of minitubers per plant was significantly lower in treatments of 10 ppm of Virazole (27.7 g), 20 ppm of Virazole (27.24 g), and 40 ppm of Salicylic acid (27.85 g) than control treatment (33.7 g).

The same data proved that, treatments of 40 ppm of Virazole (33.37 ml) and 45 Gy irradiation (32.97 ml) only increased the total volume of minitubers per plant significantly. While treatments of 30 Gy irradiation (22.72 ml) and 20

Table (32): Minitubers productivity under greenhouse conditions using in vito micotubers of virus-free plantlets of Diamont cv., developed from different treatments.

Treatment	Vegetative growth (in gm)		Total for minitubers/plant			Average of minituber	
	Fresh We.	Dry We.	Weight (gm)	Volume (ml)	Number	Weight (gm)	Volume (ml)
Control	5.33	0.63	33.7	29.80	5.25	6.42	5.68
Virazole at 10 ppm	6.42	0.66	27.7	31.94	5.50	5.04	5.81
Virazole at 20 ppm	5.64	0.61	27.24	24.07	4.25	6.41	5.66
Virazole at 40 ppm	8.03	0.63	30.10	33.37	5.75	5.23	5.80
Salicylic acid at 10 ppm	8.33	0.62	30.92	26.97	4.00	7.73	6.74
Salicylic acid at 20 ppm	5.88	0.56	32.38	29.99	4.75	6.81	6.31
Salicylic acid at 40 ppm	7.05	0.58	27.85	28.20	4.75	5.86	5.94
Irradiation at 15 Gy	5.61	0.59	31.68	30.25	5.50	5.76	5.50
Irradiation at 30 Gy	6.46	0.60	30.95	22.72	5.00	6.19	4.54
Irradiation at 45 Gy	8.66	0.60	35.17	32.97	5.00	7.03	6.59
L.S.D. at 0.05	1.51	0.04	4.29	3.12	N.S.	N.S.	N.S.

ppm of Virazole (24.07 ml) decreased it significantly if compared with control treatment (29.8 ml).

From the above mentioned results it could be concluded that, combination between tissue culture technique and chemotherapy or irradiation treatments was more beneficial with Spunta than Diamont potato cultivar. As for Spunta cv., the virus-free plants developed through tissue culture technique combined with pre-treatment with 15 Gy irradiation, 10 ppm or 40 ppm of Virazole produced the high significant increases in total fresh weight of minitubers per plant , average weight and volume of a single minituber compared with virus-free plants developed through tissue culture technique alone. In contrast, number of minituber per plant in control treatment **(Fig. 20)** was significantly larger than in the above mentioned treatments.

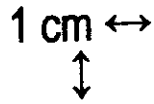


Fig. (20): Minitubers produced under greenhouse from virus-free plants of potato cultivars Spunta and Diamont developed through tissue culture technique alone (control).