RESULTS AND DISCUSSION

IV-RESULTS AND DISCUSSION

The present study aimed to investigate the suitable conditions for tissue culture technique to propagate both Mulberry and Magnolia plants. The results were divided to the following stages: -

I- Establishment stage.

II- Proliferation stage.

III- Rooting stage.

IV- Acclimatization stage.

I. Establishment stage: -

I.A. Effect of medium type, medium state, and explant type:-

I.A.1. Mulberry plants:

Almost any plant organ can be used as an explant, but the degree of success obtained will depend upon the medium and culture system used.

Comparing data of the effect of medium type in **Table (1-a), Fig. (1)** and **Photo (1)** show that necrosis of Mulberry explants significantly decreased when Murashige and Skoog medium was used as compared with the other studied nutrient media. On the other hand, Murashige and Skoog medium significantly enhanced explant development. The plant greening and medium efficiency were also significantly increased by using Murashige and Skoog medium. These results may be due the higher nitrogen content in Murashige and Skoog medium which is characterized by large concentration of nitrate (NO_3^-) and ammonium (NH_4^+) which could influence new protein synthesis in explant tissues.

Table (1): Effect of medium type, medium state and explant on the development of Mulberry explants.

Table (1-a): Effect of medium type:

Measurements	Necrosis	Explant development	Greening	Medium efficiency
Medium type:				
Murashige and Skoog	1.98 C	2.15 A	2.20 A	2.18
Woody plant medium	2.21 B	1.73 B	1.76 B	1.75
Anderson medium	3.72 A	1.67 B	1.54 C	1.61

Means of medium type followed by the same letter within each column for each category are not significantly different from each other at 1% level.

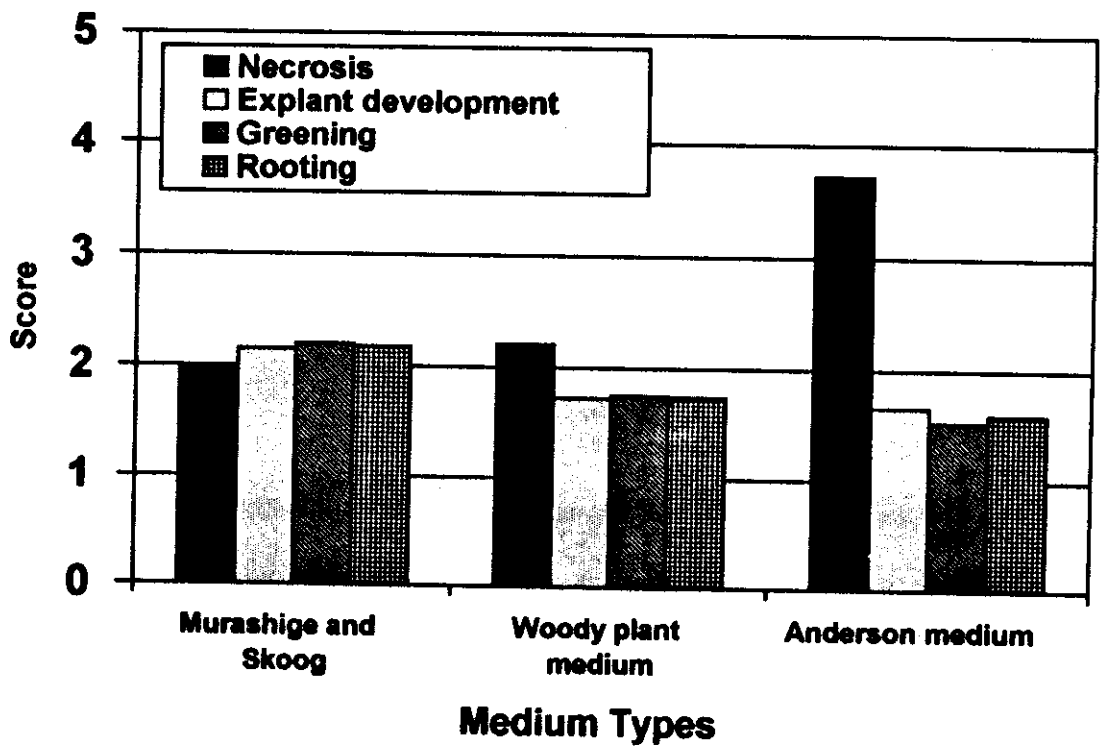

Fig. (1): Effect of medium type, medium state and explant type on the development of Mulberry explants.

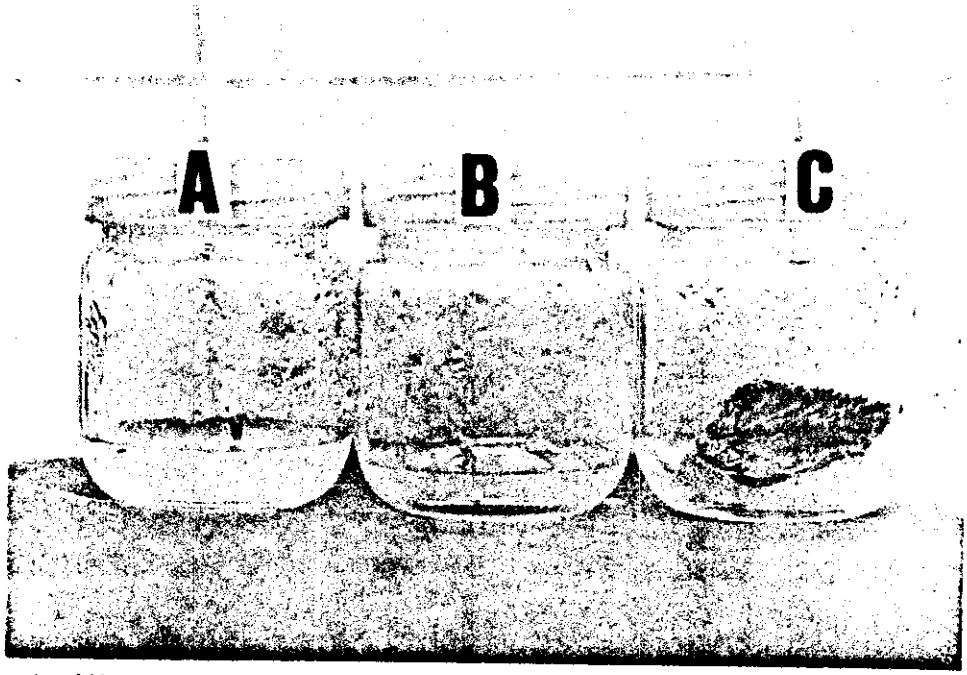

Photo (1): Effect of different medium types on explant development of Mulberry plants.

A = Murashige & Skoog **B = Woody plant medium**

C = Anderson medium

Generally, it can be said that, Murashige and Skoog medium is suitable for the establishing of Mulberry explants. These results agree with those reported by **Litz and Conover (1977)** on yucca plant, **Rugini and Devic-Verma (1982)** on almond.

Concerning the effect of medium state, **Table (1-b)** clearly indicates that the lowest necrosis was noticed with solid medium followed by liquid medium, while semi-solid medium was the worst one for necrosis. However, explant development, greening, and medium efficiency were significantly increased by using solid medium state in comparison with other M.S medium states. Meanwhile, liquid medium slightly surpassed semi-solid medium in explant development and greening without any statistical differences.

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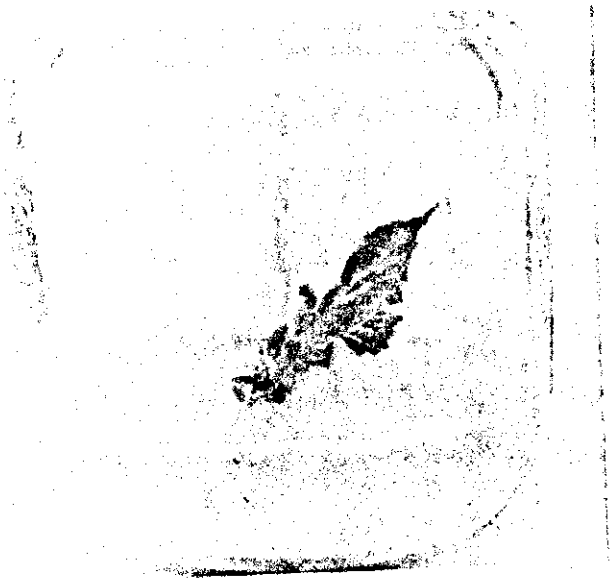
Table (1-b): Effect of medium state:

Measurements	Necrosis	Explant development	Greening	Medium State efficiency
Medium state:				
Solid	2.33 C	2.33 A	2.44 A	2.39
Semi-solid	3.03 A	1.56 B	1.35 C	1.46
Liquid	2.56 B	1.65 B	1.70 B	1.69

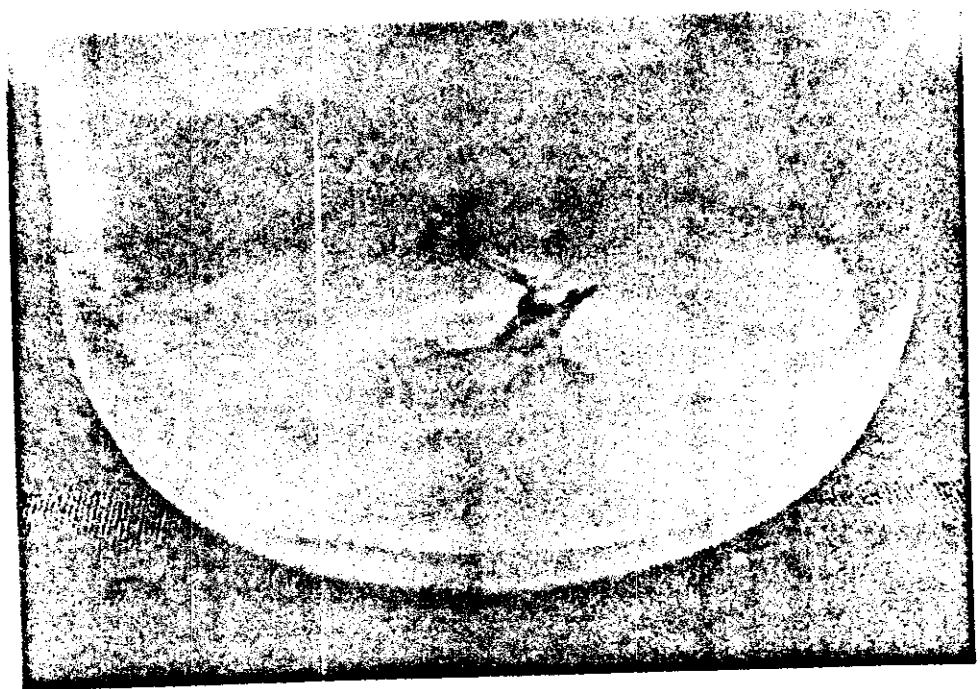
Means of medium state followed by the same letter within each column for each category are not significantly different from each other at 1% level.

Those results of medium state were, in general, agreement with the findings of **Romberger and Tabor (1971), Singha (1982), and Mackay and Kitto (1988).** They stated that agar's inhibitory action on shoot development at higher concentration has been related to a decreased uptake of medium constituents.



A

Shoot-tip



B

One-node cutting

Photo (2): Effect of explant types on explant development of Mulberry plants.

As for explant type data in **Table (1-c)** and **Photo (2)** showed that necrosis was increased when the Mulberry explant was used as one-node cutting, while necrosis was significantly decreased when shoot tips were used. On contrast, both the development of explant and greening significantly increased when Mulberry shoot-tip was used as explant. On this concern, **Tawfik (1995)** who reported that, the best number of multiple-shoots were produced from culturing of shoot-tip of Melaleuca armillaris on Murashige and Skoog medium.

Table (1-c): Effect of explant:

Measurements	Necrosis	Explant development	Greening	Medium efficiency
Explant type:				
Shoot-tip	2.41 B	2.02 A	1.98 A	2.00
One-node cutting	2.86 A	1.68 B	1.69 B	1.69

Means of explant followed by the same letter within each column for each category are not significantly different from each other at 1% level.

Concerning the effect of interaction between medium type and medium state, it could be clear from data in **Table (1-d)** that the best development of Mulberry explants occurred on solid Murashige and Skoog medium, the same trend was true with greening. While, slightly increased in both greening and explant development in relation to liquid Murashige and Skoog medium and Woody plant medium in solid state.

Table (1-d): Effect of interaction between medium type and medium state on Mulberry explants.

Measurements	Necrosis			Explant development			Greening		
Medium state	Solid	Semi solid	Liquid	Solid	Semi solid	Liquid	Solid	Semi solid	Liquid
Medium type:									
Murashige and Skoog	1.72 E	2.83 C	1.39 F	2.83 A	1.28 E	2.33 B	2.78 A	1.38 DC	2.45 B
Woody plant medium	1.83 E	2.58 C	2.22 D	2.33 B	1.33 E	1.51 DE	2.39 BC	1.28 EF	1.61 D
Anderson medium	3.45 B	3.67 B	4.06 A	1.83 CD	2.06 BC	1.11 E	2.17 C	1.39 DE	1.06 F

Means of the interaction followed by the same letter within each column for each category are not significantly different from each other at 1% level.

Table (1-e): Effect of interaction between medium type and explant type on Mulberry explants.

Measurements	Necrosis		Explant development		Greening	
Explant type	Shoot-tip	One-node cutting	Shoot-tip	One-node cutting	Shoot-tip	One-node cutting
Medium type:						
Murashige and Skoog	1.70 A	2.26 A	2.44 A	1.85 A	2.37 A	2.04 A
Woody plant medium	1.94 A	2.48 A	1.82 A	1.60 A	1.80 A	1.67 A
Anderson medium	3.59 A	3.85 A	1.78 A	1.56 A	1.70 A	1.37 A

Means of the interaction between medium type and explant type followed by the same letter within each column for each category are not significantly different from each other at 1% level.

Table (1-f): Effect of interaction between medium state and Mulberry explant types.

Measurements	Necrosis		Explant development		Greening	
Explant type	Shoot-tip	One-node cutting	Shoot-tip	One-node cutting	Shoot-tip	One-node cutting
Medium state:						
Solid	2.04 A	2.63 A	2.63 A	2.04 A	2.63 A	2.26 A
Semi solid	2.76 A	3.30 A	1.67 A	1.40 A	1.52 A	1.18 A
Liquid	2.44 A	2.67 A	1.75 A	1.55 A	1.78 A	1.63 A

Means of the interaction medium state and explant type followed by the same letter within each column for each category are not significantly different from each other at 1% level.

Table (1-g): Effect of interaction between medium type, medium state and explant type on Mulberry plant.

Measurements	Necrosis						Explant development						Greening					
Medium state	Solid		Semi solid		Liquid		Solid		Semi solid		Liquid		Solid		Semi solid		Liquid	
Explant type	Shoot tip	One-node cutting	Shoot tip	One-node cutting	Shoot tip	One-node cutting	Shoot tip	One-node cutting	Shoot tip	One-node cutting	Shoot tip	One-node cutting	Shoot tip	One-node cutting	Shoot tip	One-node cutting	Shoot tip	One-node cutting
Medium type:																		
Murashige and Skoog	1.33 A	2.11 A	2.45 A	3.22 A	1.33 A	1.45 A	3.33 A	2.13 A	1.44 A	1.11 A	2.56 A	2.11 A	2.00 A	2.56 B	1.56 EF	1.22 FGH	2.56 B	2.33 BC
Woody plant medium	1.45 A	2.22 A	2.23 A	2.89 A	2.11 A	2.33 A	2.56 A	2.11 A	1.33 A	1.33 A	1.58 A	1.44 A	2.56 B	2.22 BC	1.22 EGA	1.33 FGH	1.78 DE	1.44 EF
Anderson medium	3.33 A	3.56 A	3.56 A	3.78 A	3.89 A	4.22 A	2.00 A	1.62 A	2.22 A	1.89 A	1.11 A	1.11 A	2.33 BC	2.00 CD	1.78 DE	1.00 H	1.00 H	1.11 GH

Means of the interaction between medium type, medium state and explant type followed by the same letter within each column for each category are not significantly different from each other at 1% level.

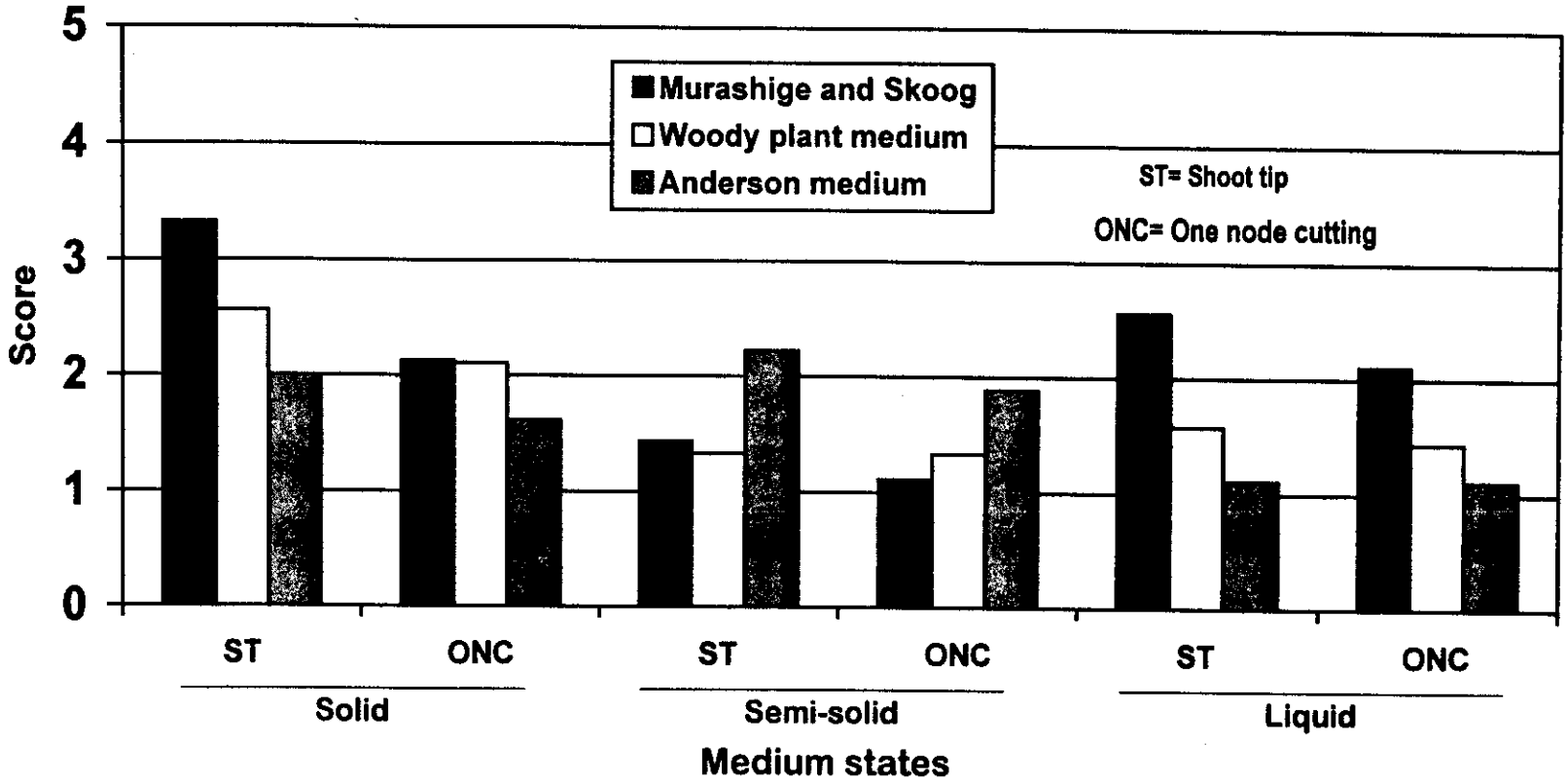


Fig. (2): Effect of interaction between medium type, medium state and explant type on the Mulberry explant development.

On the other hand, data presented in **Table (1-e)** reveal that shoot-tip or one-node cutting explants were more or less similar from statistical point of view in all studied criteria. The same reaction was also noticed between the interaction between medium state and explant type on Mulberry explants **(Table, 1-f).**

Regarding the interaction effects between medium type, medium state and explant type of Mulberry, comparing data in **Table (1-g) and Fig. (2)** clearly indicated that no significant differences on both necrosis or explant development. However, the greening recorded a significant increase when shoot-tip of Mulberry was cultured on solid Murashige and Skoog medium.

I.A.2. Magnolia plants:

The effect of medium type and medium state on the development of Magnolia explants are shown in **Table (2- a,b).** It appears from **Table (2-a) and Photo (3)** that necrosis was significantly decreased when Woody plant medium was used, while using both Nitsch & Nitsch or Lepoivre medium significantly increased the necrosis in relation to the other used media.

On the other hand, Woody plant medium significantly enhanced explant development and greening as compared with the other studied nutrient media.

Table (2-b) explains that semi-solid medium significantly increased necrosis. Meanwhile, solid medium significantly surpassed liquid media in improving both explant development and greening of Magnolia explants.

Table (2-b): Effect of medium state:

Measurements	Necrosis	Explant development	Greening	Medium State efficiency
Medium state:				
Solid	1.663 B	2.110 A	2.996 A	3.053
Semi-solid	2.997 A	1.997 B	1.663 C	1.830
Liquid	1.773 B	1.850 B	2.330 B	2.090

Means of medium type and medium state followed by the same letter within each column for each category are not significantly different from each other at 1% level.

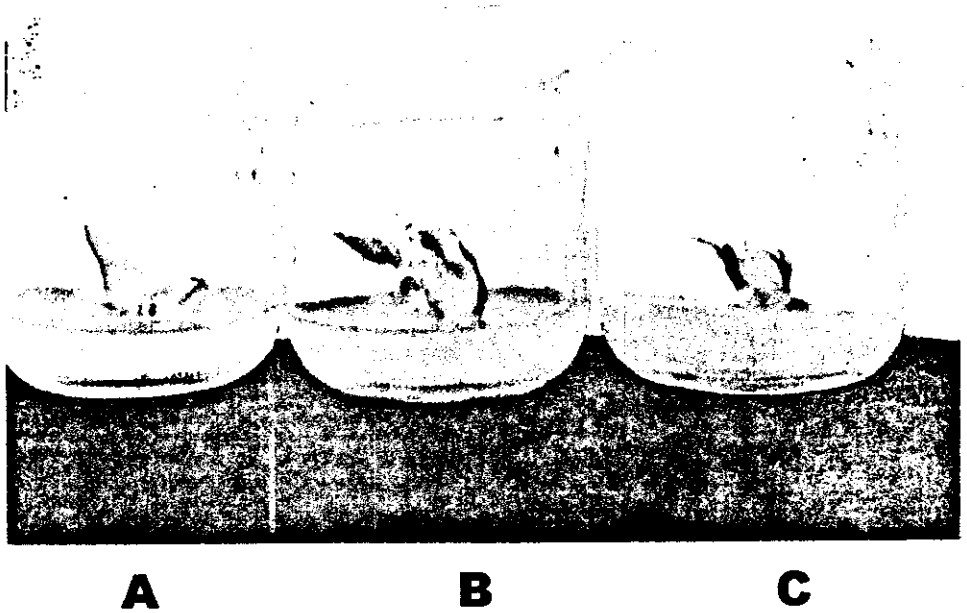


Photo (3): Effect of different medium types on development of Magnolia explant.
A = Lapoivre medium B = Woody plant medium
C = Nitsch and Nitsch medium

Table (2): Effect of medium type, medium state on the development of Magnolia explants.

Table (2-a): Effect of medium type:

Measurements	Necrosis	Explant development	Greening	Medium efficiency
Medium type:				
Woody plant media	1.553 C	3.330 A	2.887 A	3.109
Nitsch and Nitsch	2.663 A	1.663 B	1.773 C	1.718
Lepoivre	2.217 B	1.963 B	2.330 B	2.147

Means of medium type and medium state followed by the same letter within each column for each category are not significantly different from each other at 1% level.

Table (2-b): Effect of medium state:

Measurements	Necrosis	Explant development	Greening	Medium State efficiency
Medium state:				
Solid	1.663 B	2.110 A	2.996 A	3.053
Semi-solid	2.997 A	1.997 B	1.663 C	1.830
Liquid	1.773 B	1.850 B	2.330 B	2.090

Means of medium type and medium state followed by the same letter within each column for each category are not significantly different from each other at 1% level.

As for the effect of the interaction between medium type and medium state on the development of Magnolia explants, it clearly shows from **Table (2-c) and Fig. (3)** that both necrosis and greening didn't show any statistical differences when Woody plant medium or both Nitsch & Nitsch and Lepoivre media were used. However, using of Woody plant medium at solid state recorded the highest significant values for explant development of Magnolia. Generally, the above results can be summarized into using Woody plant media on solid state induced the lowest necrosis and enhanced the highest explant development, greening and medium efficiency for Magnolia explant.

Table (2-c): Effect of interaction between medium type and medium state on the development of Magnolia explants.

Measurements	Necrosis			Explant development			Greening		
Medium state	Solid	Semi solid	Liquid	Solid	Semi solid	Liquid	Solid	Semi solid	Liquid
Medium type:									
Woody plant medium	1.00 A	2.33 A	1.33 A	4.33 A	3.00 B	2.67 B	3.67 A	2.00 A	3.00 A
Nitsch and Nitsch	2.33 A	3.33 A	2.33 A	2.00 C	1.67 CD	1.33 D	2.33 A	1.33 A	1.67 A
Lapovior	1.67 A	3.33 A	1.67 A	3.00 B	1.33 D	1.56 D	3.00 A	1.67 A	2.33 A

Means of the interaction between medium type and medium state followed by the same letter within each column for each category are not significantly different from each other at 1% level.

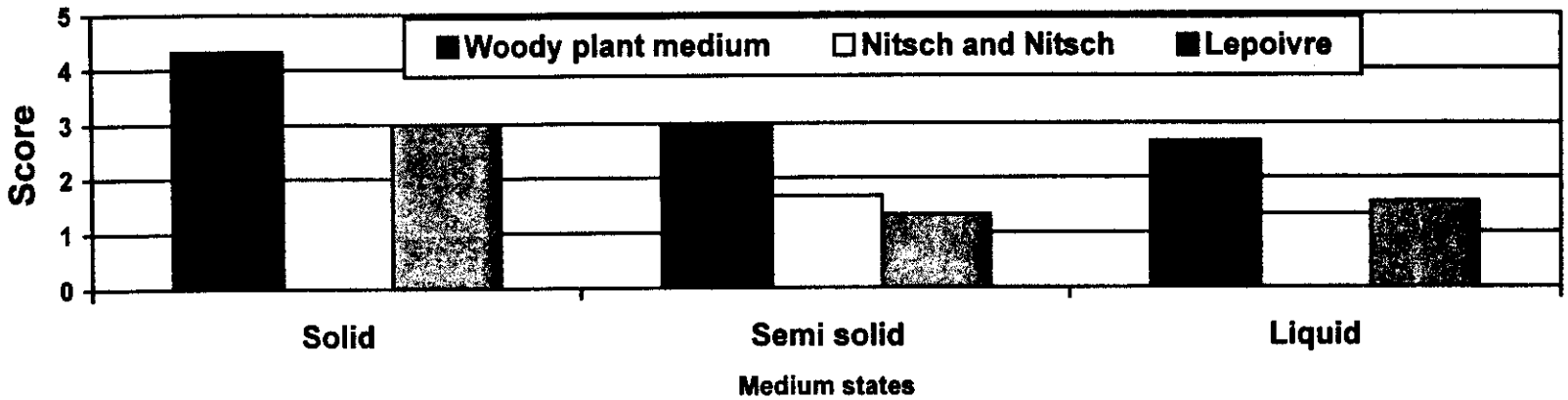


Fig. (3): Effect of interaction between medium type and medium state on the development of Magnolia explant.

I.B. Effect of explant collection date: -

I.B.1. Mulberry plants: -

Data in **Table (3) and Fig. (4)** show the effect of explant date on the development of Mulberry explants, it is clear that excised explants through both January-March and April-June gave the lowest necrosis, thus the same treatments recorded the highest values for both explant development and greening. While, the explants of October-December showed poor responses. It could be due to the seasonal variation, which sure influence the explant regeneration. There is enough evidence to indicate the effect of environmental factors such as light and temperature (**Pierik, 1967 and Gautheret, 1970**) are associated with changes in the endogenous levels of various plant growth regulators and synthesis of new ones such as the hypothetical rizocaline and phyllocaline, which act in consort with sugars in morphogenesis.

Table (3): Effect of explant date on the development of Mulberry explants with Murashige and Skoog medium.

Measurements	Necrosis	Explant development	Greening
Explant date:			
April to June	1.11 C	3.78 A	2.67 A
July to September	2.33 B	2.33 B	2.11 B
October to December	3.33 A	1.56 B	1.56 C
January to March	1.56 C	4.00 A	2.78 A

Means of explant date followed by the same letter within each column for each category are not significantly different from each other at 1% level.

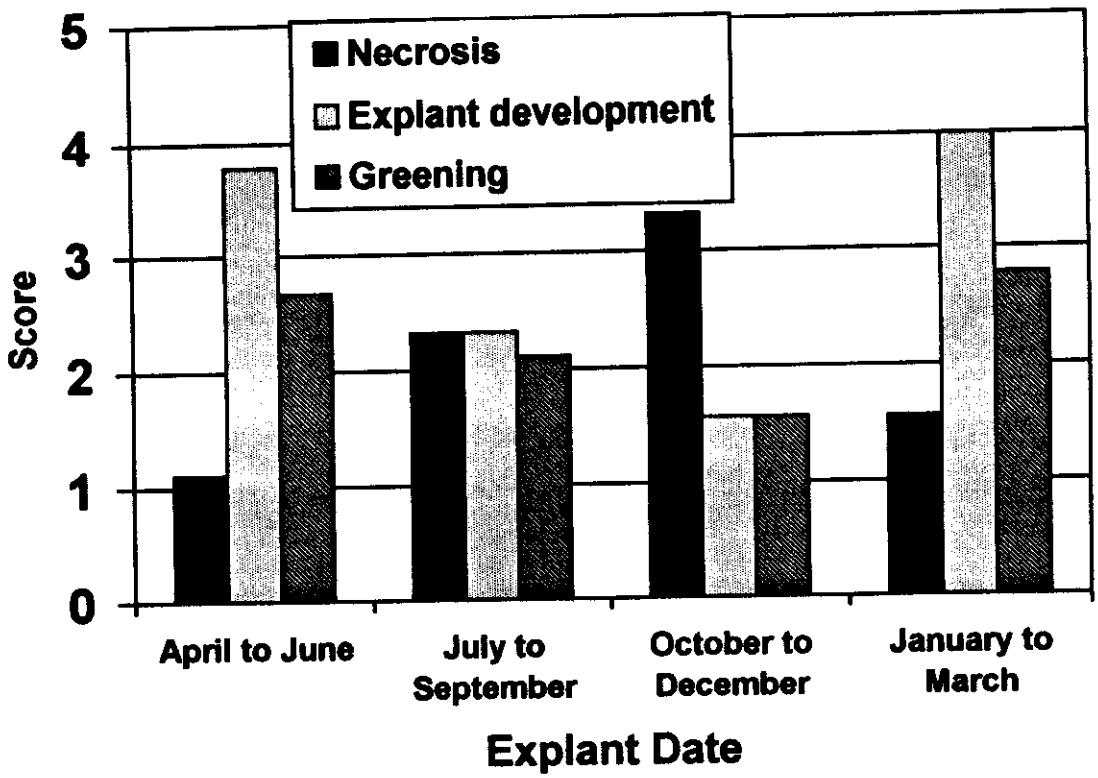


Fig. (4): Effect of explant date on the development of Mulberry explants with Murashige and Skoog medium.

I.B.2. Magnolia plants: -

The effect of explants preparation date on the development of Magnolia explants are shown in **Table (4), Fig. (5)** and **Photo (4),** the recorded data indicated that both necrosis and browning were significantly increased when the Magnolia explants were excised during September-November. This could be due to increment of the phenolic compounds, which significantly affected the explant development, greening and bud efficiency of during September to November stage. While, the explants, which excised during June-August showed the lowest values of both necrosis and browning content. Thus the explants which excised during this period (June-August) recorded the highest values of explant development,

greening and bud efficiency. On the other hand, the explants, which excised during (March-May) period gave the same responses for explant development and greening.

Table (4): Effect of explant date on the development of Magnolia explants.

Measurements	Necrosis	Browning	Explant development	Greening	Bud efficiency
Explant date:					
April to June	2.00 B	1.67 C	3.00 B	3.33 A	3.16
July to September	1.33 C	1.11 C	4.33 A	3.33 A	3.83
October to December	3.67 A	3.67 A	1.33 C	1.33 C	1.33
January to March	2.33 B	2.65 B	2.00 C	2.11 B	2.05

Means of explant date followed by the same letter within each column for each category are not significantly different from each other at 1% level.

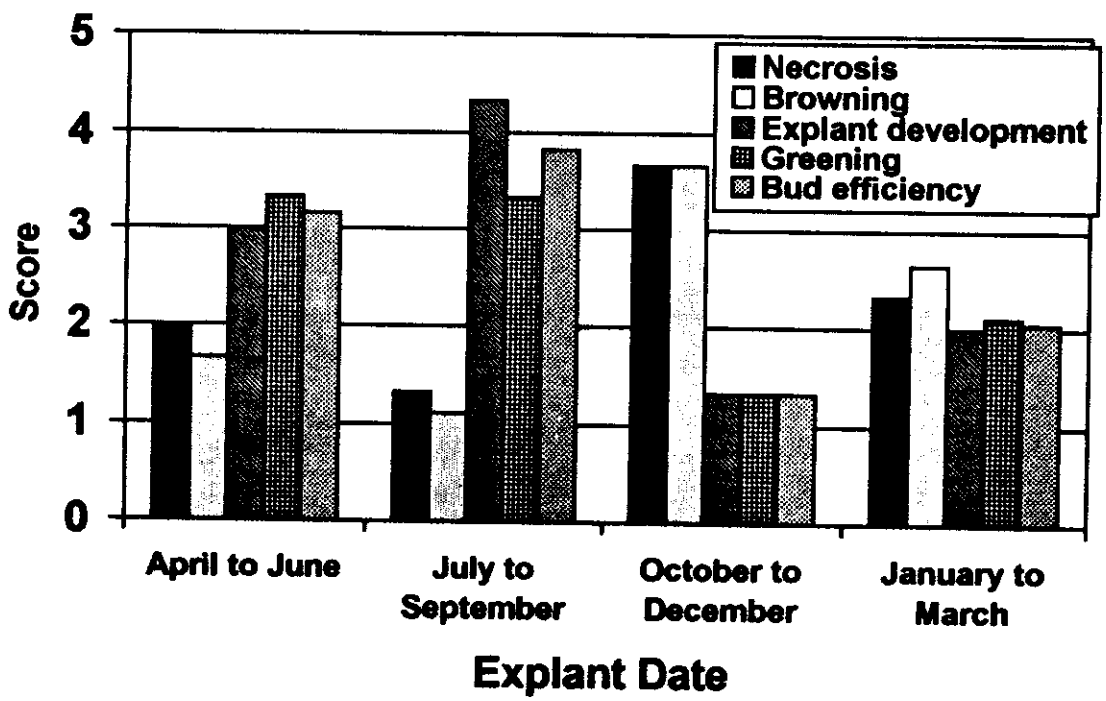
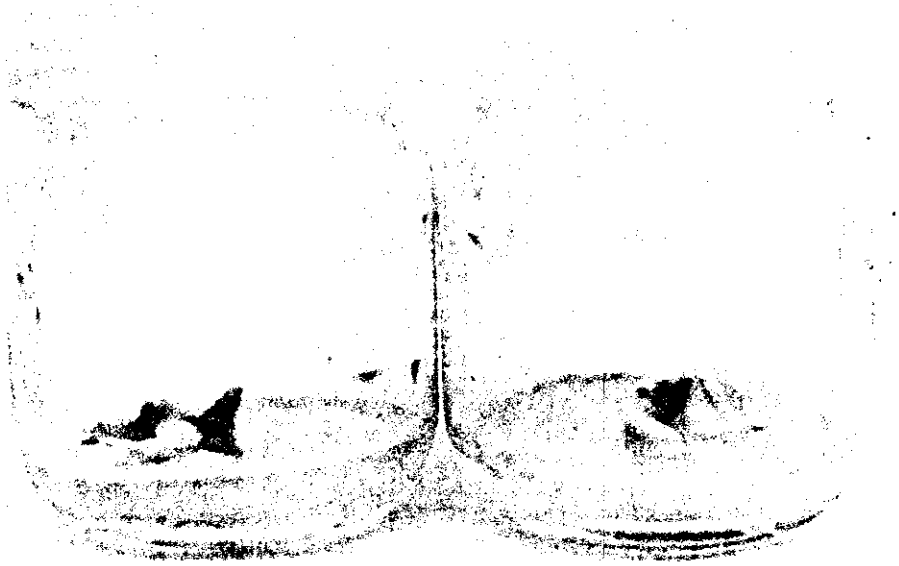
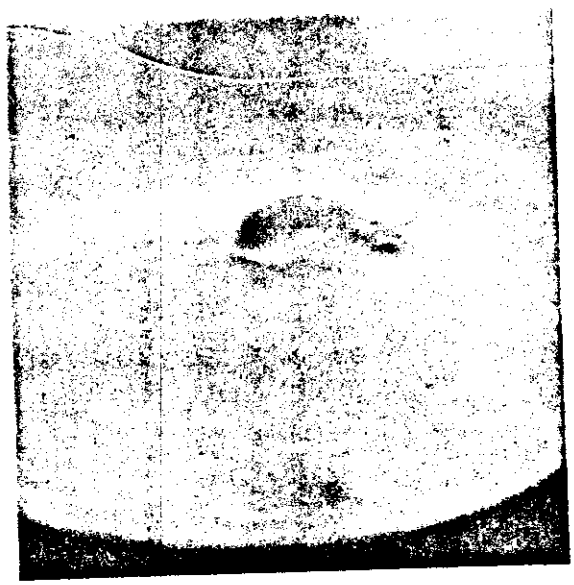


Fig. (5): Effect of explant date on the development of Mulberry explants with Murashige and Skoog medium.



April to June

January to March



October to December

Photo (4): Effect of explant date on the explant development parameters of Magnolia explant.

From the above data it could be concluded that (June-August) is the best period for excising Magnolia explants. These results are in harmony with the findings of **Fellenberg (1963)** who reported that, seasonal variations exercised a profound influence on regeneration.

I.C. Effect of anti-oxidant treatment on Magnolia explants: -

I.C.2. Magnolia plants: -

Magnolia explants contains a light concentration of phenols which always contain at least one hydroxyl group on the benzene ring **(Laomis and Battaile, 1966).** Thus, different anti-oxidant treatments were used in order to decrease the necrosis and brown color in both media and Magnolia explants. It is clear from **Table (5), Fig. (6) and Photo (5)** that anti-oxidant solution, activated charcoal and their combination induced the lowest significant decrease in necrosis as compared with the control. However, activated charcoal when added to the media, it increased both necrosis and browning. On the other hand, anti-oxidant solution significantly enhanced explant development, greening and anti-oxidant efficiency in relation to other treatments.

Meanwhile, the combination of activated charcoal and anti-oxidant solution encouraged explant development, greening and anti-oxidant efficiency. The above results indicate that anti-oxidant solution and activated charcoal or their combination succeeded in reducing the harmful effect of the accumulated phenolic compounds specially free phenolic compounds which led to minimize necrosis

and browning which in turn enhanced explant development, greening and anti-oxidant efficiency. These results agree with the findings of **Murashige (1974); Zaid and Tisseret (1983) and Zaid (1984)**. They declared that soaking the explants in ascorbic acid and citric acid or adding them to the culture medium succeeded in reducing phenolic compounds. Also, the results agreed with the findings of **Miller *et al.* (1982)** where they mentioned that the addition of ascorbic acid to the medium reduced the toxic effect of the oxidation products. **Laomis and Battaile (1966)** gave some explanation for this reaction, they reported that, several enzymes oxidize phenols of explants to quinones, where the hydroxyl group is oxidized leading to the formation of quinone and water, the brown color that frequently develops in cell cultures is due to the formation of these quinones that are well known to be toxic and are inhibitory to plant cellular growth.

Table (5): Effect of different antioxidant treatments on the development of Magnolia explants.

Measurements	Necrosis	Browning	Explant development	Greening	Antioxidant efficiency
Antioxidants:					
Control	4.67 A	4.33 A	1.00 C	1.00 C	1.00
Antioxidant solution	3.33 B	3.00 B	2.67 A	2.67 A	2.67
Active charcoal	3.67 B	3.33 B	1.33 C	1.33 BC	1.33
Ant. sol + Act. ch.	3.33 B	1.67 C	2.00 B	1.67 B	1.87

Means of antioxidant treatments followed by the same letter within each column for each category are not significantly different from each other at 1% level.

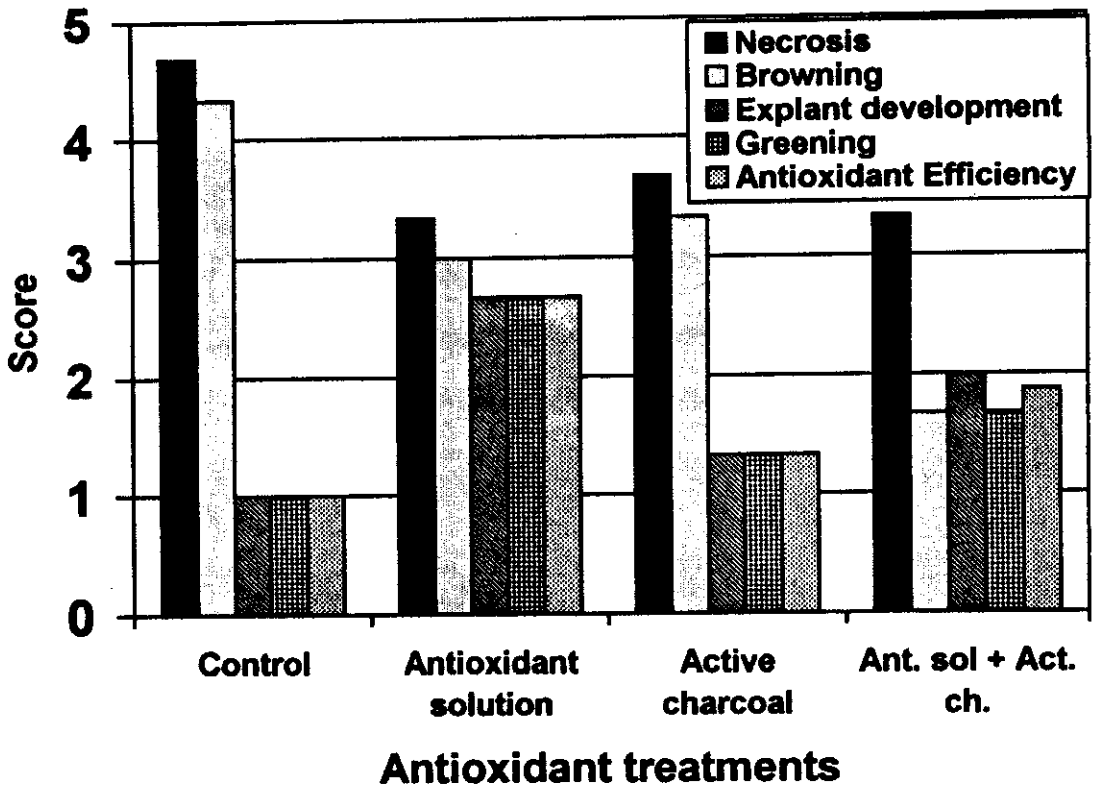
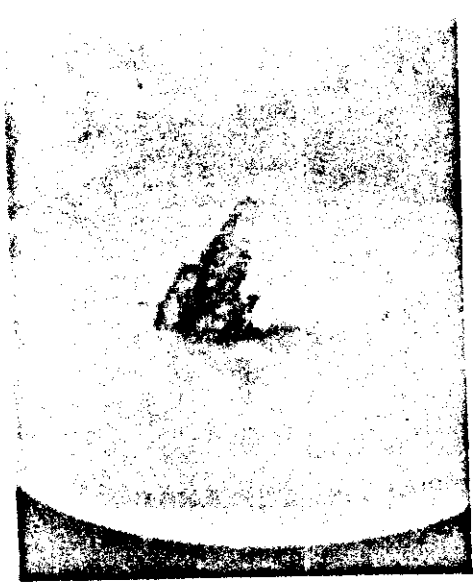


Fig. (6): Effect of different antioxidant treatments on the development of Magnolia explants.

On the other hand, activated charcoal had a harmful effect on explant development and this may be due to the adsorption of both phenolic compounds and other growth promoters on charcoal particles. This is in accordance with the finding of **Pierik (1987)** who assured this assumption. Also, agreed with the findings of **Preip and Emgelhart (1977)** where they stated that activated charcoal inhibited several shoot formations in azalea plants when added to the culture medium at the establishment stage.



Control

Antioxidant solution

Combination of antioxidant + activated

Photo (5): Effect of different antioxidant treatments on the development of Magnolia explant.

I.D. Effect of terminal bud size: -

I.D.2. Magnolia plants:

Data presented in **Table (6)**, **Fig. (7)** and **Photo (6)** explain the effect of the Magnolia terminal bud size on explant development. It is clear that choosing the largest buds in Woody plant medium significantly enhanced both explant development and greening more than the other bud sizes. On the other hand, the smaller terminal bud size the lowest value of both explant development and greening. In the same time, the medium bud size was intermediate in both explant development and greening. However, necrosis didn't show any statistical differences between the three sizes of the buds.

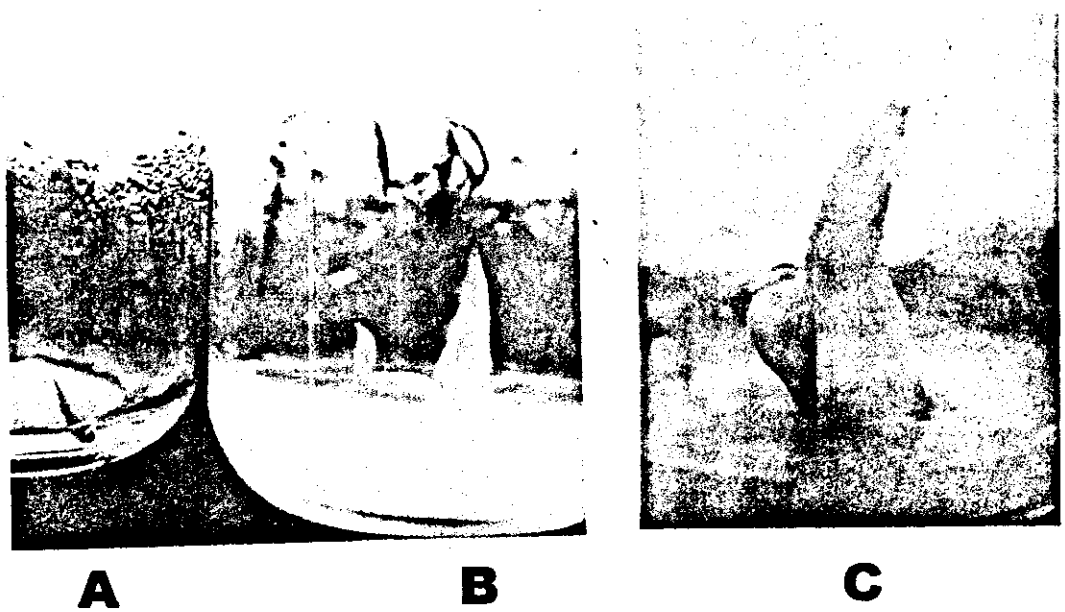


Photo (6): Illustrate different terminal buds sizes of Magnolia at culturing time.

Table (6): Effect of Magnolia terminal bud size on the development of explants.

Measurements	Necrosis	Explant development	Greening
Bud size:			
Large	2.33 A	4.33 A	3.00 A
Medium	3.00 A	2.33 B	2.00 B
Small	2.33 A	1.33 C	1.00 C

Means of terminal bud sizes followed by the same letter within each column for each category are not significantly different from each other at 1% level.

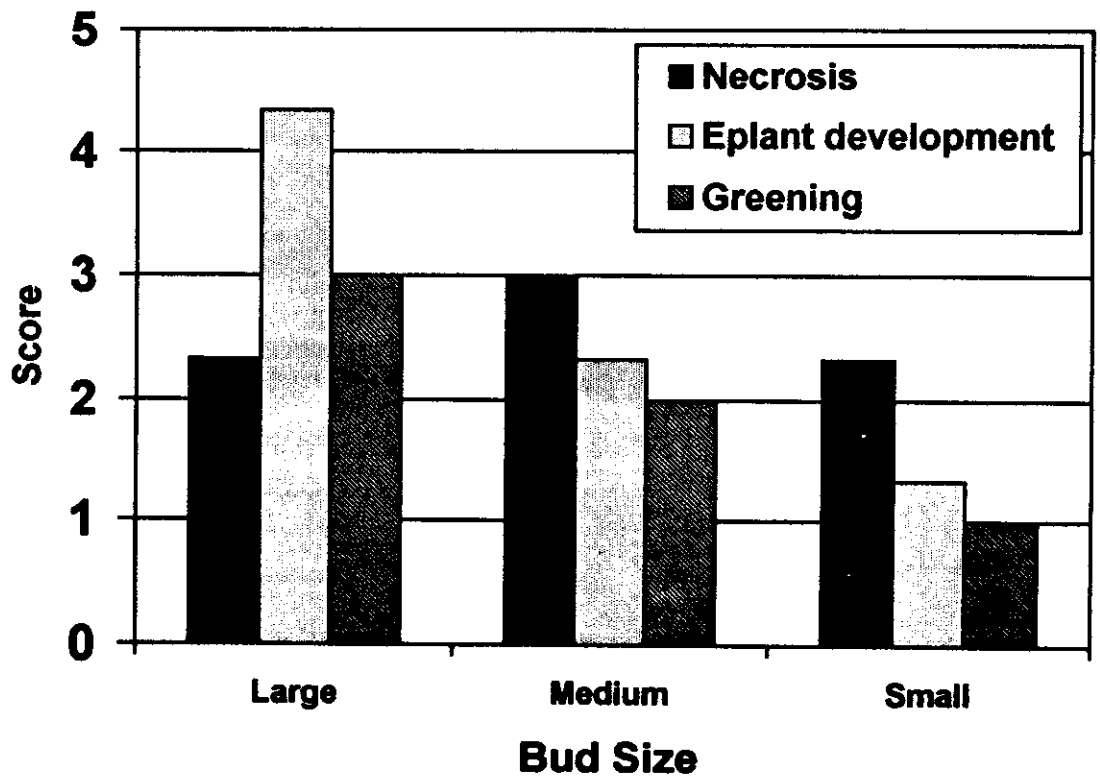


Fig. (7): Effect of Magnolia terminal bud size on the development of explant

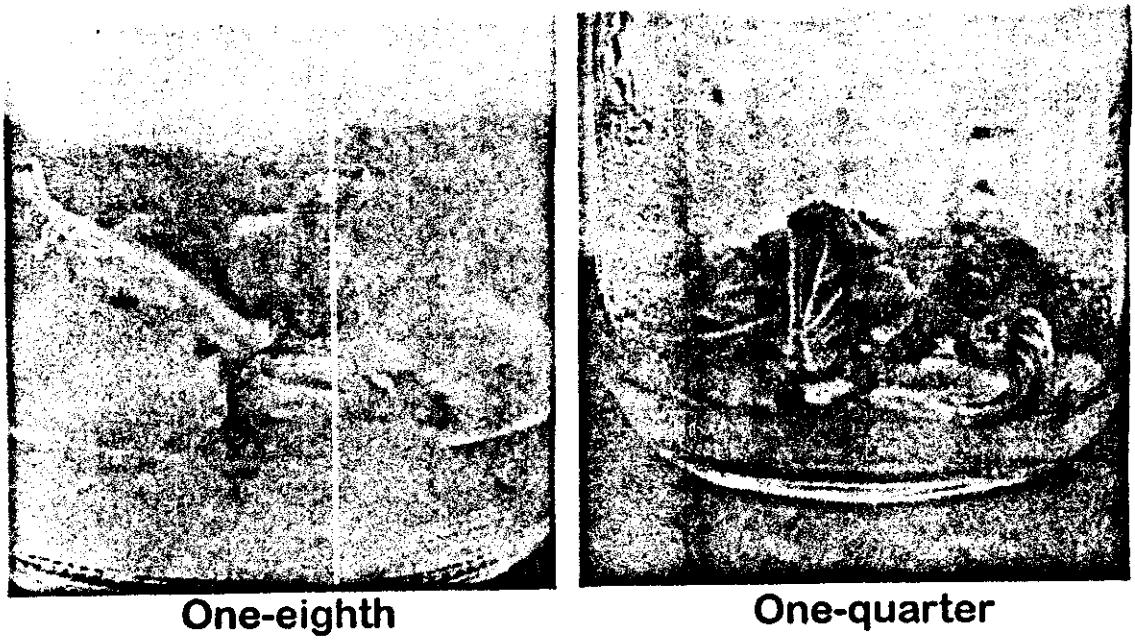
Generally, the above results can be summarized into culturing of the largest terminal bud size produced the highest values for explant development and greening. It could be due to the change on the hormonal status of buds. In this concern, Altman and Goren (1971) found that sprouting of buds in vitro was affected by regulators in a similar way to bud in vivo. Sprouting was retarded by IAA and ABA in the medium, but slightly enhanced by GA3.

I.E. Effect of medium strengths: -

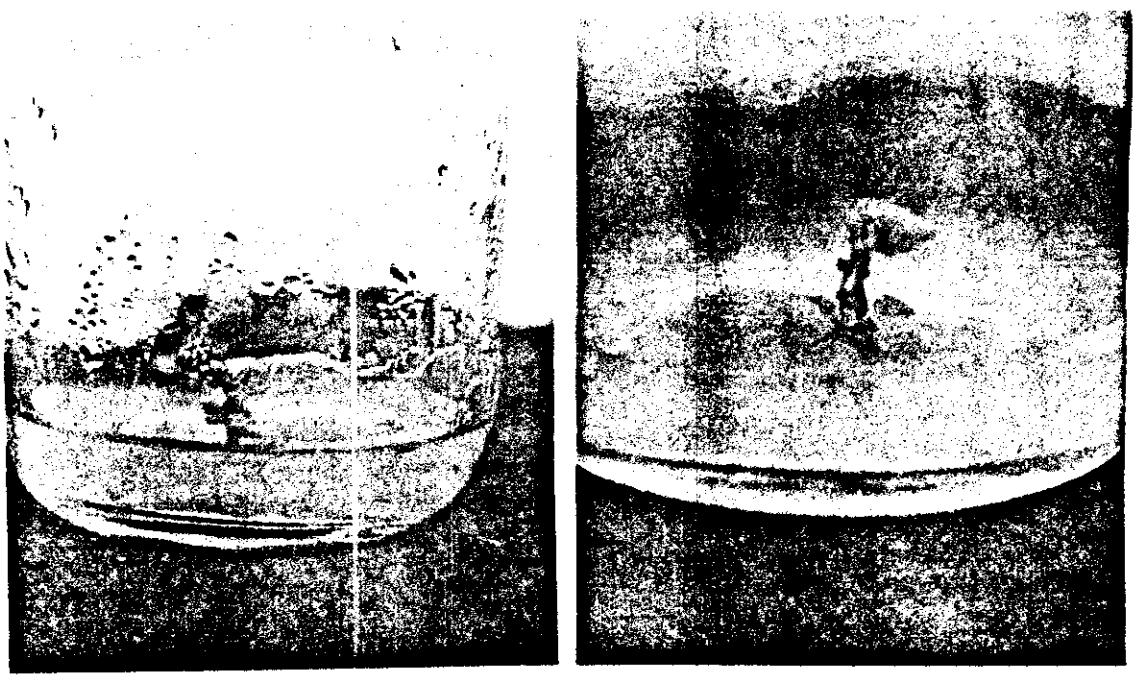
I.E.1. Mulberry plants:

The effects of Murashige and Skoog medium salts strength (full, one-half, one-fourth and one-eighth) on necrosis, explant development and greening on Mulberry explants are shown in **Table (7), Fig. (8) and Photo (7)** the recorded data indicated that continuous decrease in medium strength up to one-fourth and one-eighth resulted in a significant reduction in necrosis in relation to full and one-half medium strengths. While, both treatments recorded slight differences in both explant development and greening without any significancy. However, full and one-half strength significantly increased the explant development and greening as compared with other medium strengths.

In conclusion, it seems that, full and one-half medium strengths gave the best results. Similar conclusions were reported by **Barghchi and Alderson (1983 & 1985) and Yang & Ludders (1994).** They recommended one-half strength Murashige and Skoog medium for enhancing shoot formation of pistachio plants.



One-eighth One-quarter



One-half Full

Photo (7): Effect of different medium strengths on explant development of Mulberry plants.

I.E.2. Magnolia plants:

The effect of Woody plant media salts (full, one-half, one-quarter and one-eighth) on necrosis, explant development and greening of *Magnolia grandiflora* explants are shown in **Table (8), Fig. (9) and Photo (8).**

Comparing the effect of woody plant medium salts strengths, the data recorded indicated that the highest value of explant development was obtained with using full medium strength. However, a decrease in medium strength to one-half resulted in a significant reduction in explant development and greening as well as further decreases to, one-fourth and one-eighth medium strengths.

In general, full medium strength gave the best results for the explant development and greening. Thus, full medium strength of woody plant medium can be recommended for increasing the development of Magnolia explants. In this connection, **Freet (1987)** on Salvia gregii, found that full and half strength of MS salts were superior to quarter strength of MS salts.

Table (8): Effect of different woody plant medium strengths on the development of Magnolia explants.

Measurements	Necrosis	Explant development	Greening
Medium strengths:			
Full	1.67 A	3.67 A	2.67 A
One-half	1.33 A	2.33 B	2.33 B
One-quarter	1.33 A	1.56 C	1.67 C
One-eighth	1.11 A	1.33 C	1.67 C

Means of medium strengths followed by the same letter within each column for each category are not significantly different from each other at 1% level.

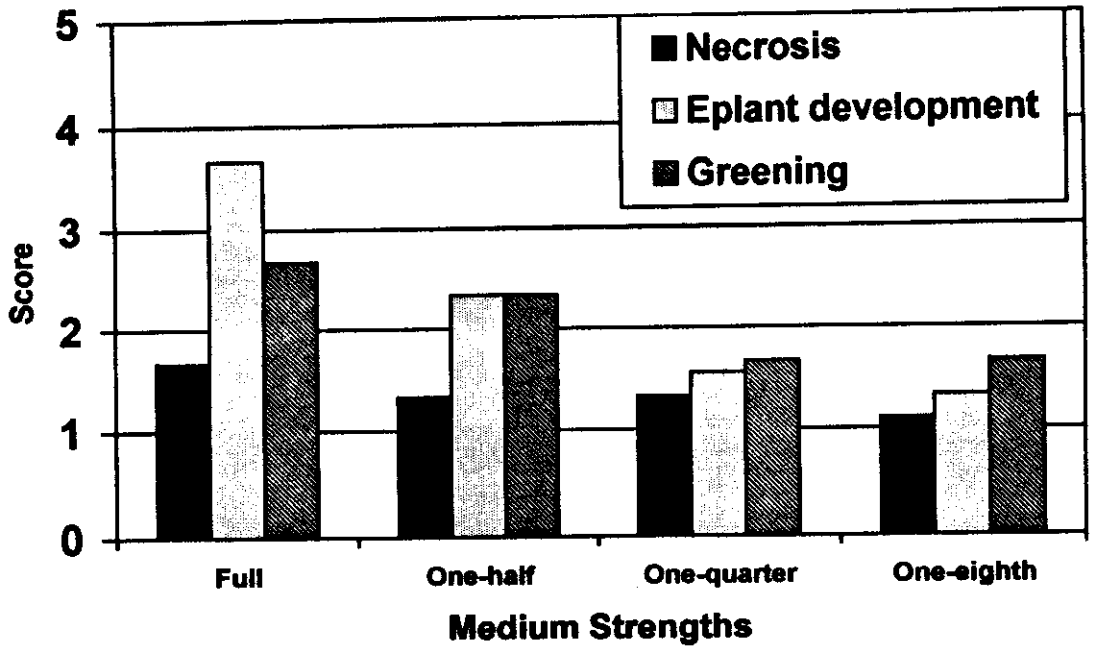


Fig. (9): Effect of different woody plant medium strengths on the development of Magnolia explants.

I.F. Growth curve:-

I.F.2. Magnolia plants:-

The growth curve of Magnolia explant **Fig. (10)** clearly showed that break of bud from shoot tips occurred after 3 days from culturing of the explant (lag phase). Then the growth start slow for 9 days (log phase). Then followed with a rapid increase in growth for 27 days from culturing time (progressive phase). The stationary phase started after 27 days from culturing time. Thus, subculturing should be done after 27 days for Magnolia plants. These results are in agreement with the findings of **Zaied, (1997)** who reported that proliferation curve of stone fruit passed through four phases (log,

lag, progressive, and stationary phases) and reached the peak of proliferation after 28 days. Also, she added that subculturing should be done after 28 days and before starting of stationary phase.

Different stages of apical meristem of Magnolia explant development during establishment stage were shown in **Photo (9).**

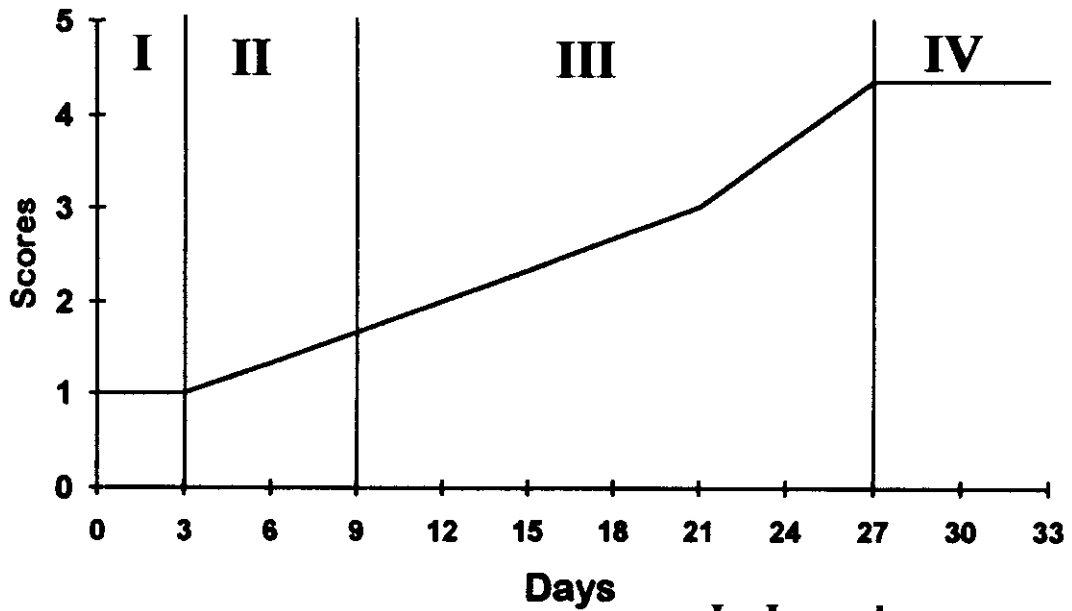
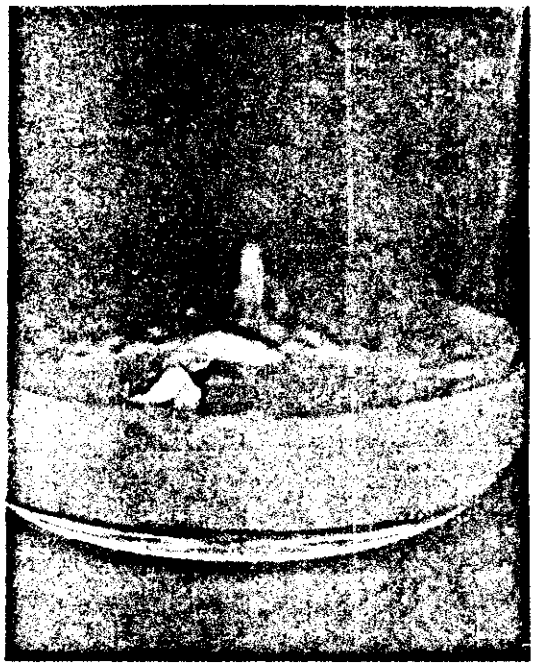
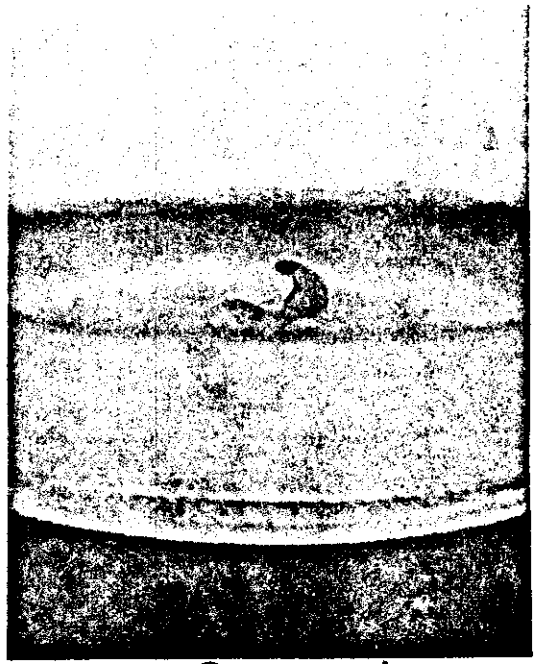


Fig. (10): Growth curve of Magnolia plants

Photo (10) showed the different apical bud sizes of Magnolia before culturing on the medium at establishment stage. However, **Photo (11)** indicates the apical bud development from starting to the end of establishment stage.



One-eighth



One-quarter

One-half

Full

Photo (8): Effect of different medium strengths on development of apical Magnolia bud.

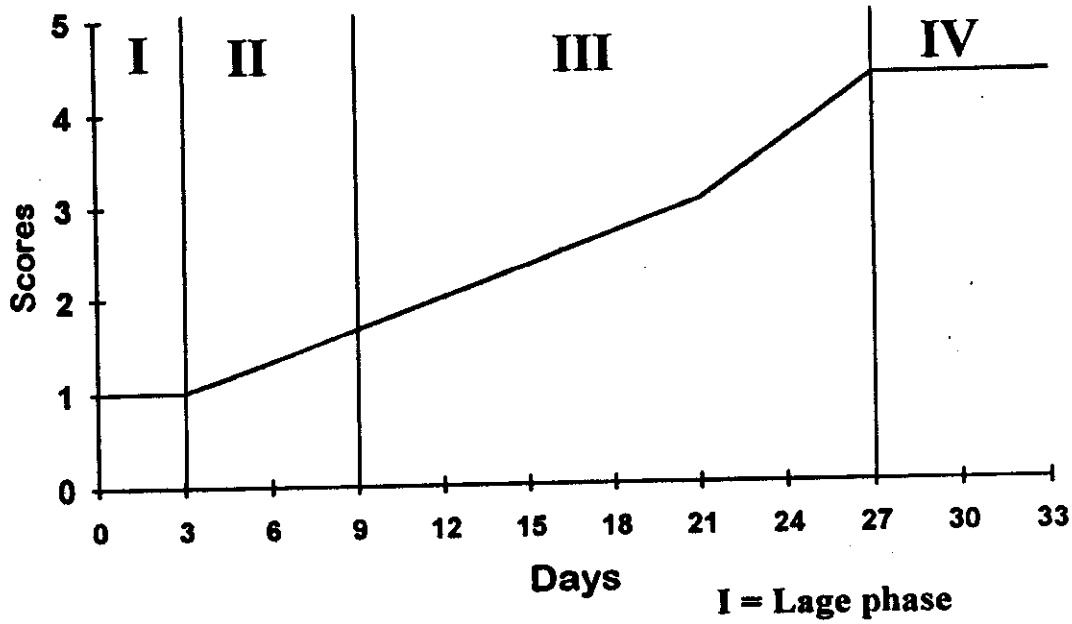


Fig. (10): Growth curve of Magnolia plants

Photo (9) showed the different apical bud sizes of Magnolia before culturing on the medium at establishment stage. However, **Photo (10)** indicates the apical bud development from starting to the end of establishment stage.

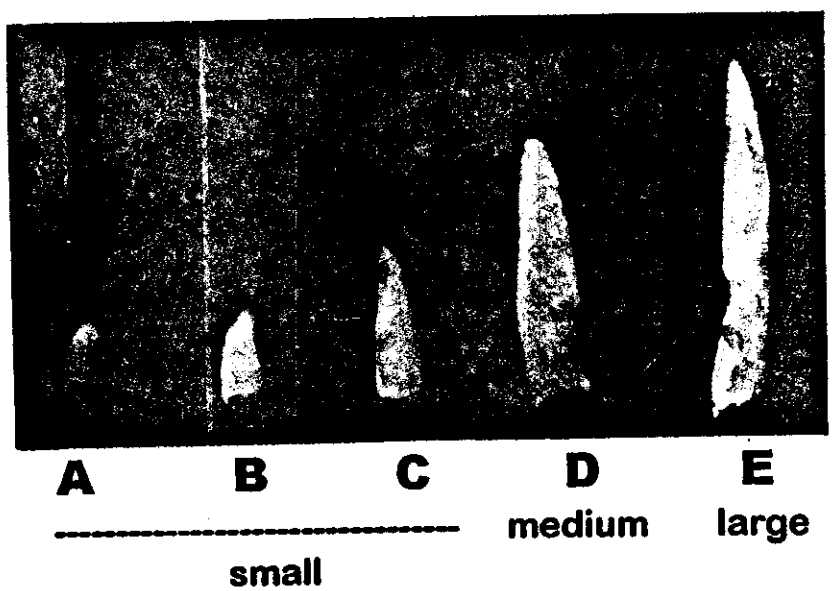


Photo (9): Different apical bud sizes of Magnolia

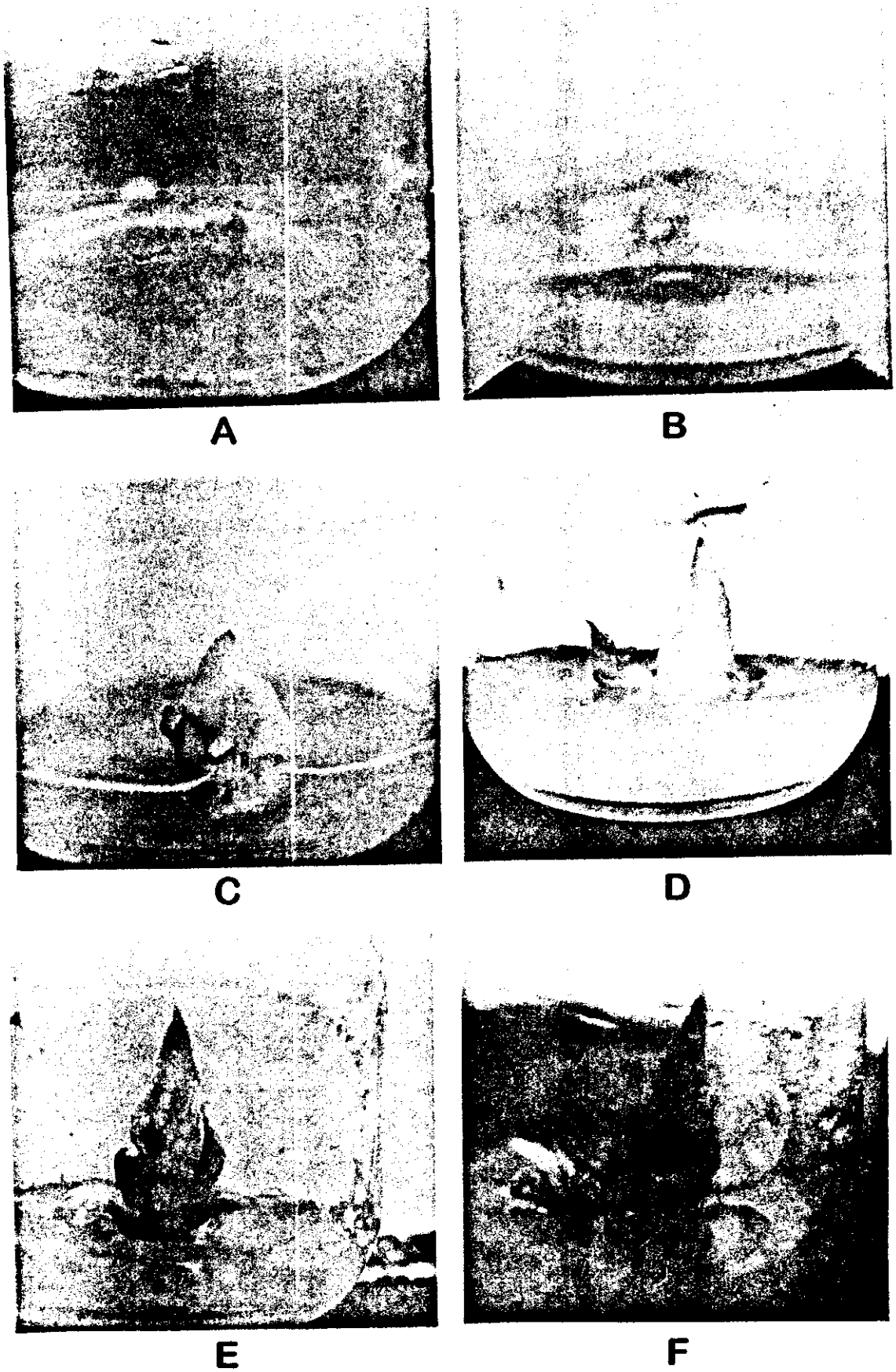


Photo (10): Development stages of apical bud of Magnolia plants.

II. Proliferation Stage:-

The important goal on this stage is rapid multiplication of Mulberry plantlets by using either medium state, cytokinin types or concentrations which are the most important factors affecting this stage.

II.A. Effect of medium state : -

The effect of Murashige and Skoog medium state on the growth and proliferation of Mulberry explants are shown in **Table (9)** and **Fig. (11).** It is found that both solid and liquid medium states succeeded in reducing necrosis, while semi-solid medium significantly increased necrosis and proliferation. On the other hand, both solid and liquid medium significantly stimulated the shoots development, while the responses to development were the lowest values with semi-solid medium. The same trend was true with greening, where both liquid and solid medium recorded the highest values of greening.

Generally, the above results can be summarized that using Murashige and Skoog medium in either solid or liquid state increased the development with more greening, while semi-solid state significantly increased the proliferation. These results are, in general, agreement with the findings of **Emam (1997)** found that semi-solid medium increased necrosis and proliferation of some grape and strawberry cultivars, while liquid and solid medium improved growth and chlorophyll.

Table (9): Effect of Murashige and Skoog medium state on the growth and proliferation parameters of Mulberry explants.

Measurements	Necrosis	Shoots development	Proliferation	Greening
Medium state:				
Solid	1.78 B	2.67 A	3.33 A	3.33 AB
Semi-solid	3.22 A	1.78 A	4.00 A	2.89 B
Liquid	2.00 B	2.33 A	2.33 B	3.78 A

Means of medium state followed by the same letter within each column for each category are not significantly different from each other at 1% level.

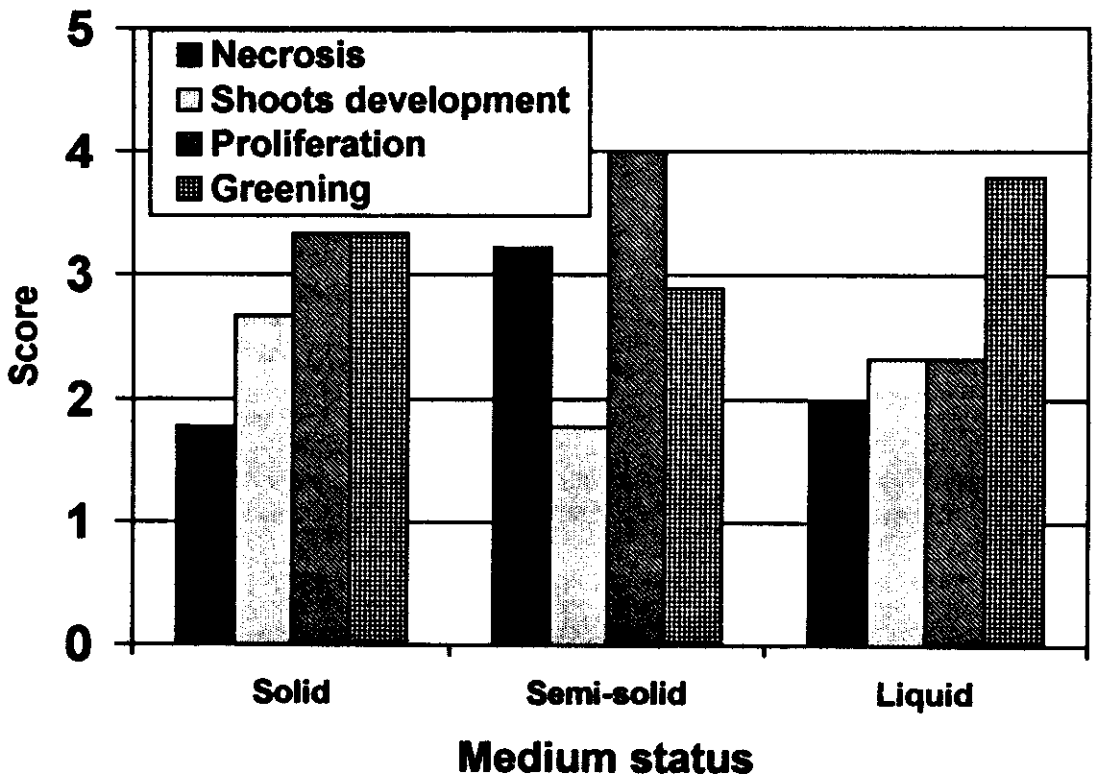


Fig. (11): Effect of Murashige and Skoog medium state on the growth and proliferation parameters of Mulberry explants.

II.B. Effect of cytokinin types and cytokinin like: -

Table (10) and Fig. (12) deal with the effect of some cytokinin types and cytokinin-like substance on growth and proliferation parameters of Mulberry explants. It is clear that kinetin. significantly decreased the necrosis as compared with both other cytokinin types or cytokinin-like. At the same time, both shoots development and greening significantly increased as kinetin was added to the culture medium. On the other hand, addition of BAP significantly enhanced proliferation in relation to thidiazuron at same level.

From all the above results, it could be concluded that kinetin reduced necrosis and improved both shoots development and greening, while zeatin and BAP increased the proliferation. These results go in line with the findings of **Hansen and Lazarte (1984) and Lazarte (1984),** they recommended BAP for increasing proliferation of pecan. Also, these results are in agreement with the findings of **Brokowska and Opilowska (1988)** on sour cherry, and **Hagagy (1992)** on Williams banana.

Table (10): Effect of different cytokinin types and cytokinin concentration on the growth and proliferation parameters of Mulberry explants.

Measurements	Callus	Necrosis	Shoots development	Proliferation	Greening
Conc. (2mg/L.)					
Kinetin	1.00 D	1.44 C	3.33 A	1.78 C	4.00 A
Zeatin	1.78 C	2.00 B	2.56 B	2.67 B	3.67 AB
BAP (6-benzylaminopurine)	2.33 B	2.11 B	2.33 B	3.56 A	3.00 B
Thidiazuron	3.11 A	4.56 A	1.11 C	1.33 C	1.33 C

Means of cytokinin types followed by the same letter within each column for each category are not significantly different from each other at 1% level.

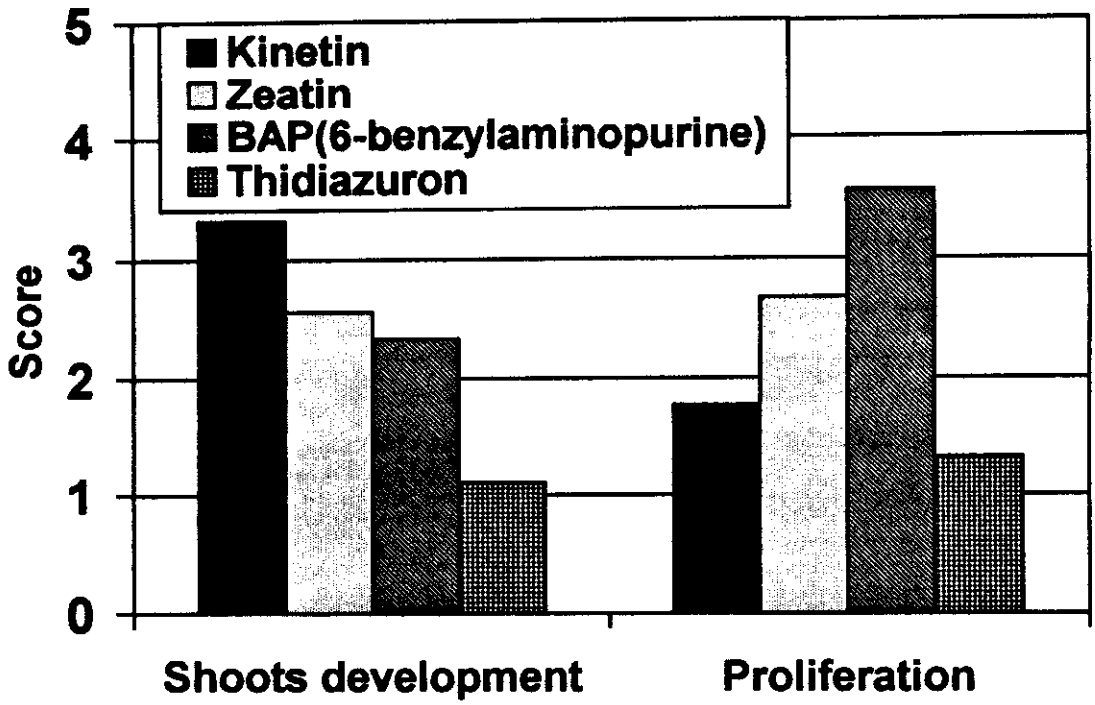
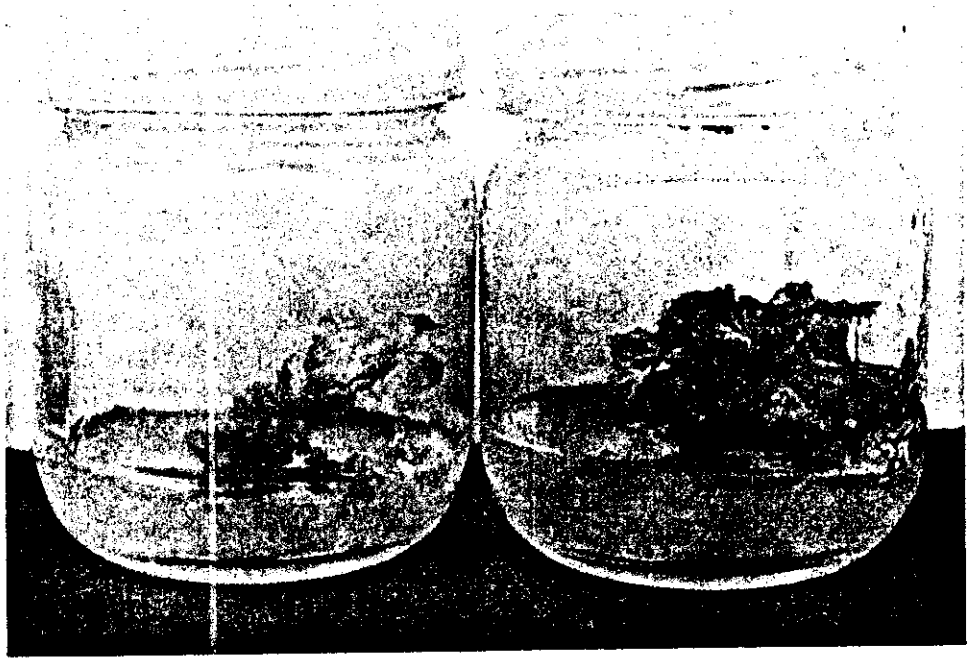


Fig. (12): Effect of different cytokinin types and cytokinin-like on the growth and proliferation parameters of Mulberry explants.

II.C. Effect of different concentrations of BAP (6-benzyl amino purine): -

Data in **Table (11), Fig. (13) and Photo (12)** show the effect of different concentrations of BAP on the growth, development and proliferation of Mullberry explants. It could be noticed that the increase of BAP the concentration to 4 mg/L. was significantly increased both necrosis and callus formation, while the lowest concentration (0.05 mg/L.) recorded the lowest values for necrosis and callus.



0.5 mg/L. 1.0 mg/L.

2.0 mg/L. 4.0 mg/L.

Photo (11): Effect of different 6-benzylaminopurine on proliferation of Mulberry explants.

On the other hand, the concentration of 1 mg/L. of BAP recorded the highest values for both shoots development and greening, while, 2 mg/L. recorded the highest values of proliferation.

Generally, lower concentration of BAP reduced necrosis, while improved the shoots development and greening. However, higher concentration increased the proliferation. Meanwhile, higher concentration induced adverse effect on development and greening. These results are in harmony with the findings of Wood (1982) who found that combination of 4 mg/L.BAP and 1 mg/L. IBA was the most effective on shoot proliferation of pecan. Coleman and Ernst (1990) indicated that the greatest number of axillary shoots of Populus deltoides occurred when 1 mg/L. zeatin was used, while 0.25 mg/L. produced the greatest growth as compared to the other concentration. Hagagy (1992) stated that using 2 mg/L. BAP induced the highest proliferation, while the lower concentration of cytokinins greatly increased growth of William's banana and higher concentration of thidiazuron up to 4 - 6 mg/L. killed the culture plantlets.

Table (11): Effect of different concentration of BAP (6benzylamino purine) on the growth, development and proliferation of Mulberry explants.

Measurements	Callus	Necrosis	Shoots development	Proliferation	Greening
BAP treatments:					
0.50 mg/l.	1.00 C	1.11 C	1.78 B	1.11 D	3.11 B
1.00 mg/l.	1.22 C	1.44 BC	3.56 A	2.11 C	3.78 A
2.00 mg/l.	2.33 B	1.89 B	1.89 B	4.00 A	2.00 C
4.00 mg/l.	3.33 A	3.00 A	1.78 B	3.33 B	1.44 D

Means of concentration followed by the same letter within each column for each category are not significantly different from each other at 1% level.

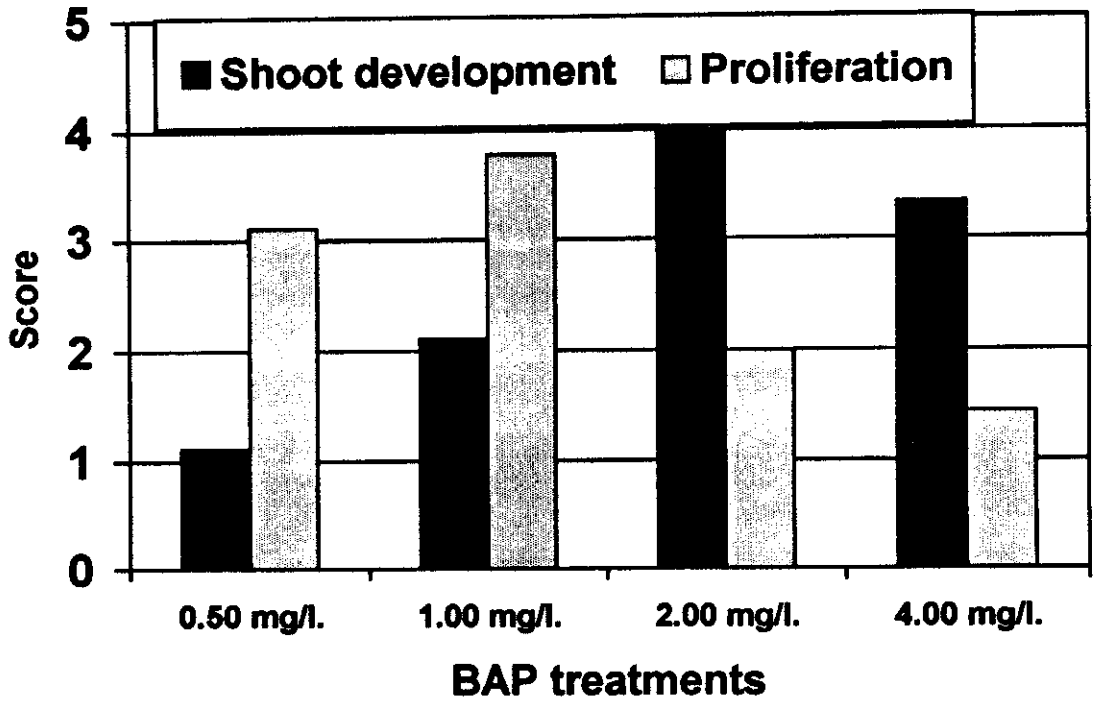


Fig. (13): Effect of different concentration of BAP (6benzyl amino purine) on shoot development and proliferation of Mulberry explants.

III. Rooting stage: -

III.1. Shoot elongation:-

III.1.A. Effect of different concentrations of GA_3: -

Table (12), Fig. (14) and Photo (13) show the effect of different concentrations of gibberellic acid (GA_3) on shoot elongation and greening of Mulberry explants. It is clear that, shoot length was increased significantly with increasing GA_3 concentration up to 2.0 mg/L. as compared with the lower concentrations of GA_3 i.e. 0.0, 0.5 and 1.0 mg/L. On the other hand, 1.0 mg/L. and 2.0 mg/L. GA_3 concentrations significantly improved greening followed by control as compared with 0.5 mg/L. and 0.4 mg/L. GA_3 concentrations.

0.5 mg/L. 1.0 mg/L.

2.0 mg/L. 4.0 mg/L.

Photo (12): Effect of different GA_3 concentrations on shoot elongation of Mulberry explants.

Generally, the results show that 2 mg/L. GA3 induced the longest shoot length with good greening content. These results, in general, agreement with the findings of **Zayed (1997)** and **Mohamed (1997).** They stated that shoot length of stone fruits plants and nut fruit plants increased greatly by using either 2 or 4 mg/L. GA_3.

Table (12): Effect of different concentration of GA_3 (gibberellic acid) on the shoot elongation and greening of Mulberry explants.

Measurements	Shoot length	Greening
GA_3 treatments:		
Control	1.00 C	2.67 AB
0.50 mg/l.	1.89 BC	2.44 B
1.00 mg/l.	2.56 ABC	3.22 A
2.00 mg/l.	3.67 A	3.33 A
4.00 mg/l.	3.33 AB	2.33 B

Means of GA_3 concentration followed by the same letter within each column for each category are not significantly different from each other at 1% level.

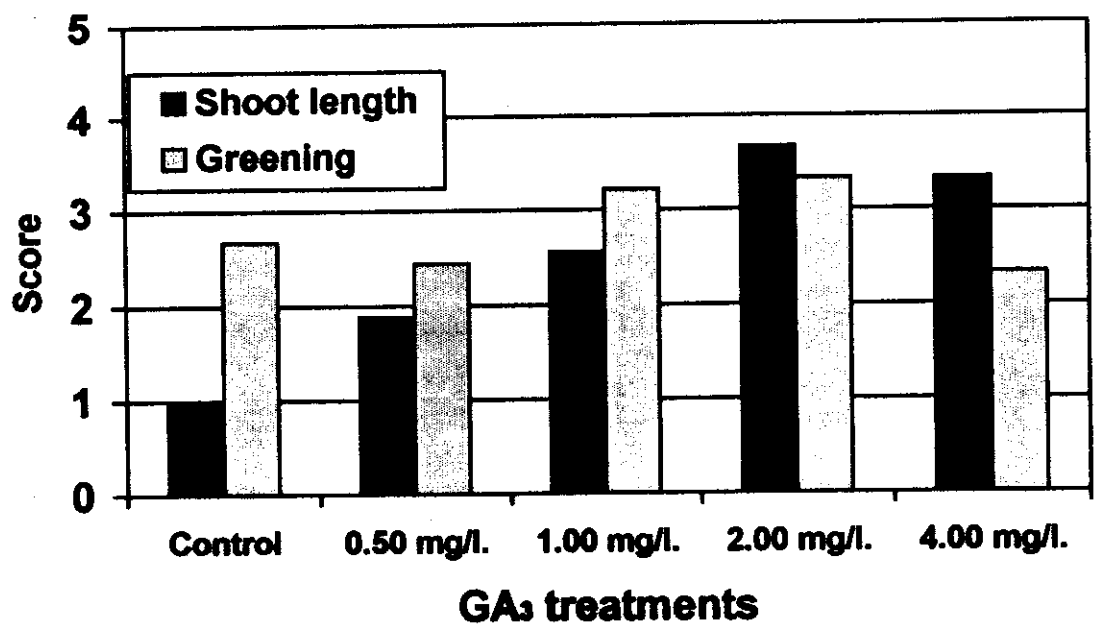


Fig. (14): Effect of different concentration of GA_3 (gibberellic acid) on the shoot elongation and greening of Mulberry explants.

III.2. Root formation: -

III.2.A. Effect of medium state: -

Regarding the effect of different states of woody plant medium on growth and rooting of Mulberry plantlets it is clear from **Table (13)** and **Fig. (15)** that solid and liquid medium states significantly reduced necrosis in comparison with semi-solid medium. On the other hand, solid and liquid medium states significantly increased development and greening as compared with semi-solid medium. Moreover, liquid medium significantly improved rooting as compared with the other used medium states.

At all events, one can conclude that liquid and solid medium states increased shoots development and greening, while liquid medium improved rooting. These results in harmony with the findings of **Choudhary (1991)** on carnation; **Rothore *et al.* (1992)** on zizuphus (*Z. mauritiana*) and **Mohamed (1997)** on pistachio. They stated that best rooting was occurred in liquid medium.

Table (13): Effect of different medium state of woody plant medium on the shoot development and rooting of Mulberry explants.

Measurements	Necrosis	Shoots development	Greening	Rooting
Medium state:				
Solid	1.78 B	2.69 A	3.67 A	2.44 B
Semi-solid	3.22 A	1.89 B	2.11 B	1.67 C
Liquid	2.00 B	3.00 A	3.78 A	4.11 A

Means of medium state followed by the same letter within each column for each category are not significantly different from each other at 1% level.

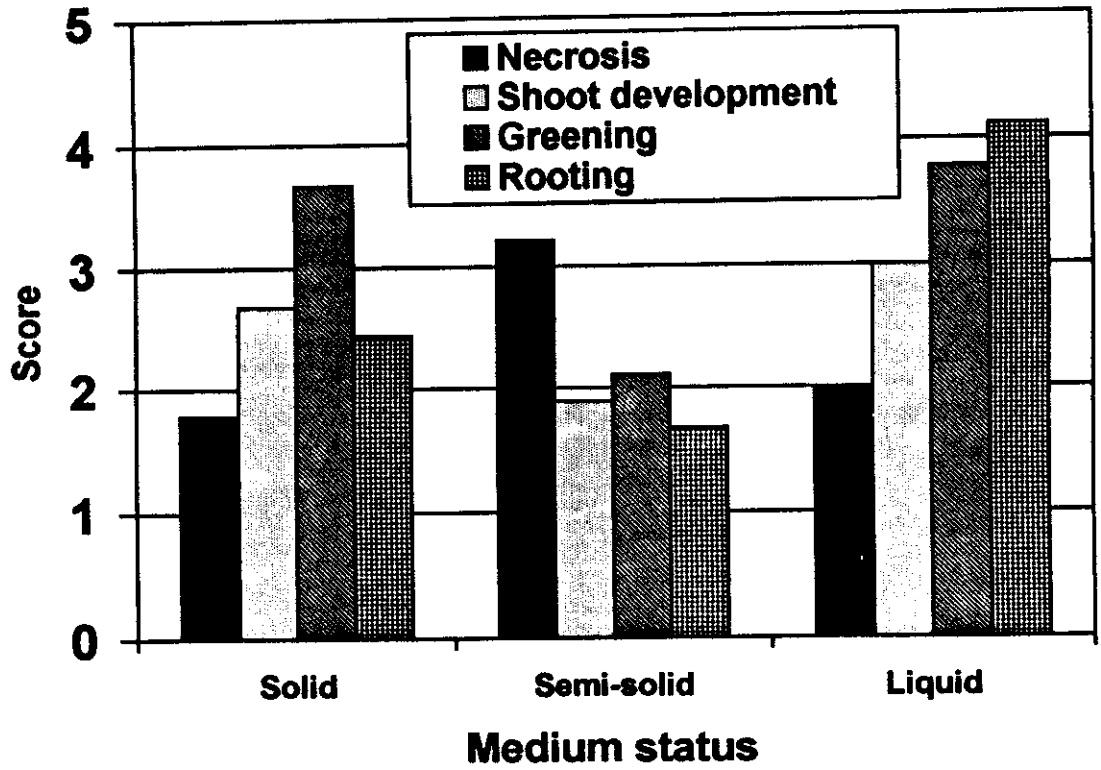


Fig. (15): Shows effect of different medium status of woody plant medium on growth and rooting of Mulberry plantlets.

III.2.B. Effect of auxin type and concentration: -

Referring to the effect of different auxin types on rooting of Mulberry plantlets. It is appear from **Table (14-a)** and **Photo (14)** that, callus and necrosis decreased significantly by IAA and NAA as compared with IBA. However, IAA induced highly significant increase in shoots development and greening over the other used treatments. On the other hand, rooting increased significantly by IBA application.

Regarding the effect of auxin concentration on rooting as shown in **Table (14-b) and Photo (15).** It is obvious that 4.0 mg/L.

was significantly increased callus and necrosis then followed by 2.0 mg/L. as compared with the lower concentrations (0.5 and 1.0 mg/L.). However, the lower concentrations i.e. 0.5 and 1.0 mg/L. profoundly increased shoots development and greening. On the other hand, 2.0 mg/L. increased rooting with high significancy. Dealing with the interaction between auxin type and concentration as shown in **Table (14-c)** and **Fig. (16)** show that 1.0 mg/L. auxin concentration induced a significant increase in shoots development over other combination. However, 2.0 mg/L. of IBA enhanced significant increase in rooting over other combinations. On the other hand, 4.0 mg/L. IBA induced significant increase of callus production over auxin concentrations for all auxin types. Generally, it is easy to conclude that IBA was superior than IAA and NAA in rooting promotion. Meanwhile, high concentrations of auxin (2.0 mg/L.) induced the best rooting. These results go in line with the findings of **Kim *et al.* (1985)** who reported that IBA effectively induced Mulberry root formation at all tested concentrations. Meanwhile, the aforementioned results somewhat agree with the finding of **Resoti *et al.* (1980).** They mentioned that the best rooting occurred for Japanese plum when 2 or 4 mg/L. IBA was added to the modified Murashige and Skoog medium.

Table (14): Effect of different auxin types and different concentration on the growth and rooting parameters of Mulberry plantlets.

Table (14-a): Effect of different auxin types:

Measurements	Callus	Necrosis	Shoots development	Greening	Rooting
Treatment:					
IAA	1.53 B	1.53 B	2.92 A	3.67 A	2.20 C
IBA	2.14 A	2.17 A	2.64 B	3.08 B	3.03 A
NAA	1.19 B	1.75 B	2.59 B	3.20 B	2.69 B

Table (14-b): Effect of different concentrations:

Measurements	Callus	Necrosis	Shoots development	Greening	Rooting
Concentration (mg/L.) :					
0.50	1.00 C	1.00 D	2.85 B	3.74 A	1.63 D
1.00	1.18 C	1.37 C	3.45 A	3.82 A	2.22 C
2.00	2.08 B	2.04 B	2.71 B	3.07 B	3.59 A
4.00	2.89 A	2.85 A	1.85 C	2.63 C	3.11 B

Means of auxin types followed by the same letter within each column for each category are not significantly different from each other at 1% level.

Table (14-c): Effect of interaction between auxin type and concentration.

Measurements	Callus			Necrosis			Explant development			Greening			Rooting		
Auxin type	IAA	IBA	NAA	IAA	IBA	NAA	IAA	IBA	NAA	IAA	IBA	NAA	IAA	IBA	NAA
Concentration:															
0.5 mg/l.	1.00 D	1.00 D	1.00 D	1.00 A	1.00 A	1.00 A	3.44 A	2.78 B	2.33 BC	4.00A	3.44A	3.75A	1.56 H	1.78 H	1.56 H
1.0 mg/l.	1.00 D	1.33 D	1.22 D	1.11 A	1.67 A	1.33 A	3.33 A	3.56 A	3.45 A	4.33A	3.65A	3.56A	1.89 GH	2.56 EF	2.28 FG
2.0 mg/l.	1.78 C	1.56 B	1.89 C	1.67 A	2.44 A	2.00 A	2.78 B	2.67 B	2.67 B	3.33A	2.89A	3.00A	2.44 F	4.44 A	3.89 B
4.0 mg/l.	2.33 B	3.67 A	2.67 B	2.33 A	3.55 A	2.67A	2.11 CD	1.56 E	1.89 DE	3.00A	2.44A	2.45A	2.89 DE	3.33 C	3.11 CD

Means of each parameter with the same letter within each column for each category are not significantly different from each other at 1% level.

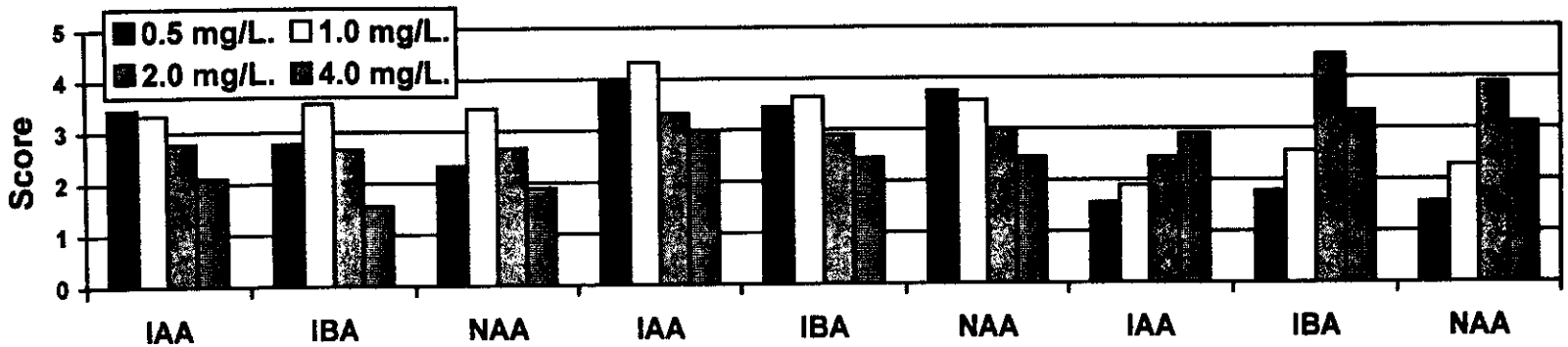


Fig. (16): Effect of interaction between auxin type and concentration.

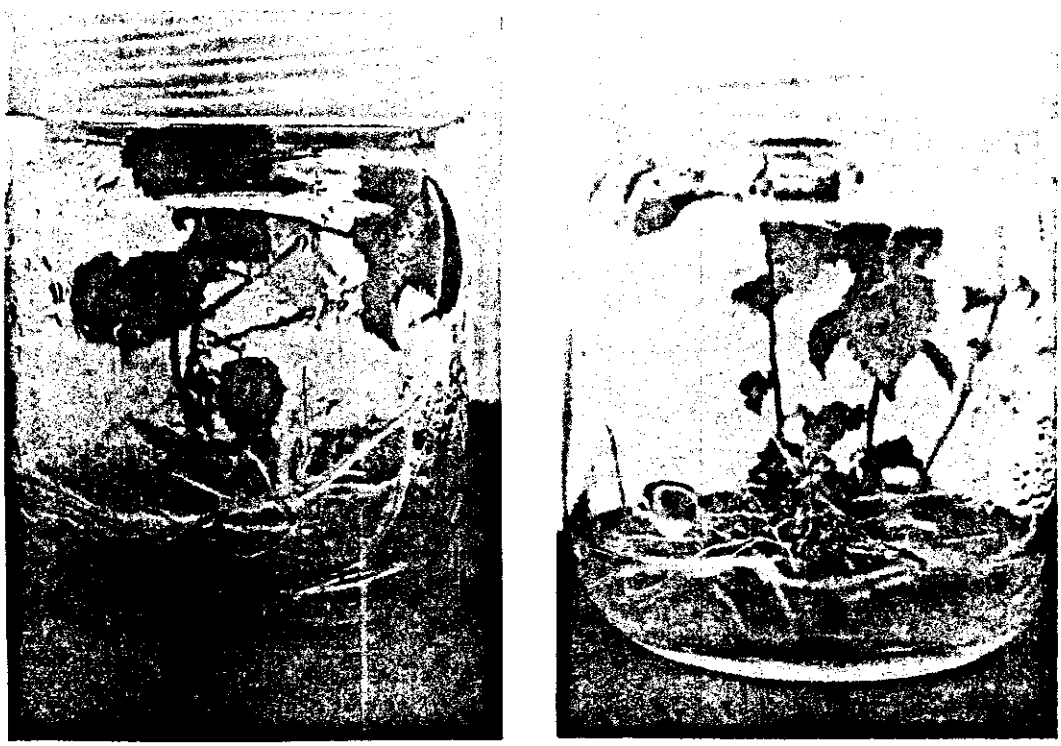
A B C

Photo (13): Effect of different auxin types on growth of Mulberry plants.

A = IAA B = IBA C = NAA

Regarding the effect of auxin concentration on rooting as shown in **Table (14-b) and Photo (14).** It is obvious that 4.0 mg/L. was significantly increased callus and necrosis then followed by 2.0 mg/L. as compared with the lower concentrations (0.5 and 1.0 mg/L.). However, the lower concentrations i.e. 0.5 and 1.0 mg/L. profoundly increased shoots development and greening. On the other hand, 2.0 mg/L. increased rooting with high significancy. Dealing with the interaction between auxin type and concentration as shown in **Table (14-c)** and **Fig. (16)** show that 1.0 mg/L. auxin concentration induced a significant increase in shoots development over other combination. However, 2.0 mg/L. of IBA enhanced significant increase in rooting over other combinations. On the other hand, 4.0 mg/L. IBA induced significant increase of callus production over auxin concentrations for all auxin types. Generally, it is easy to conclude that IBA was superior than IAA and NAA in

0.5 mg/L. 1.0 mg/L.



2.0 mg/L. 4.0 mg/L.

Photo (14): Effect of different auxin concentrations on growth and rooting of Mulberry plants.

IV. Acclimatization:

IV.A. Effect of different agricultural media:

It is clear from **Table (15) and Fig. (17)** that using of the combination treatment of (33% sand + 33% peat-moss + 33% loam) succeeded in increasing the percentage of survival to the highest level (93%) then followed with combination treatment (50% sand + 50% loam), (50% sand + 50% peat-moss) and finally (50% peat-moss + 50% loam) where the percentage of survival reached 73, 54 and 46%, respectively. Also, the data clarified that using of any of the agricultural media alone in acclimatized stage had an adverse effect on survival.

Table (15): Effect of different agriculture media treatments on survival of Mulberry plant during acclimatization stage.

Treatments	Survival
Sand at the rate of 100%	5.00
Peat moss at the rate of 100%	4.00
Loam at the rate of 100%	6.00
50% sand + 50% peat moss	54.00
50% sand + 50% loam	73.00
50% peat moss + 50% loam	46.00
33% sand + 33% peat moss + 33% loam	93.00

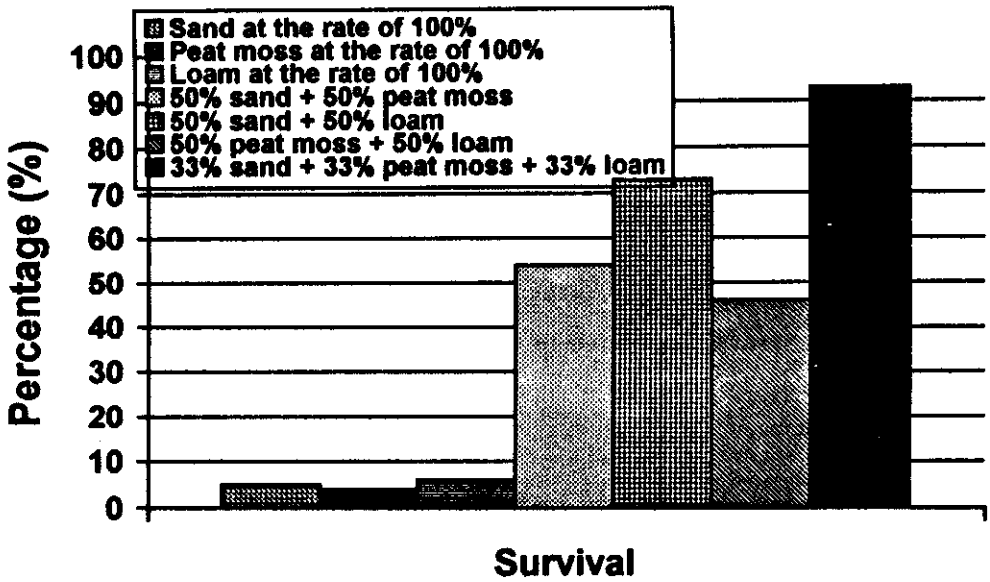


Fig. (17): Effect of different agriculture media on survival of Mulberry plant.

In general, the above mentioned results reflect that the survival of Mulberry plants in a combination treatment (sand + peat-moss + loam) reached to 93%. These results agreed with the findings of **Jain *et al.* (1990) and Yadav *et al.* (1990).** They stated that the percentage of Mulberry survival reached 89% and 75%, respectively.

IV.B. Behavior of plantlets growth during different acclimatization phases:

IV.B.1. Laboratory phase:

It is clear from **Table (16-a), Fig. (18) and Photo (16)** that observed growth parameters under investigation i.e. plant length, number of shoots, average shoot length, number of green leaves, and growth index were slowly increased during the first 3 weeks of the gradual adaptation (laboratory acclimatization). Meanwhile, a

noticeable increase was clear during the other three weeks (4th, 5th, and 6th weeks) specially both number of green leaves and growth index parameters.

Table (16): Effect of different phases during acclimatization stage on the growth parameters of acclimatized Mulberry plants.

Table (16-a): Laboratory phase:

Reading Date of	Plant length (cm)	Number of shoots	Average shoot length	Number of green leaves	Growth index
Week (1)	75	17	48	91	57.75
Week (2)	85	19	70	152	81.50
Week (3)	100	21	76	230	106.75
Week (4)	125	24	84	310	135.75
Week (5)	150	27	93	386	164.00
Week (6)	175	31	104	425	183.75
Week (7)	200	35	124	450	202.25

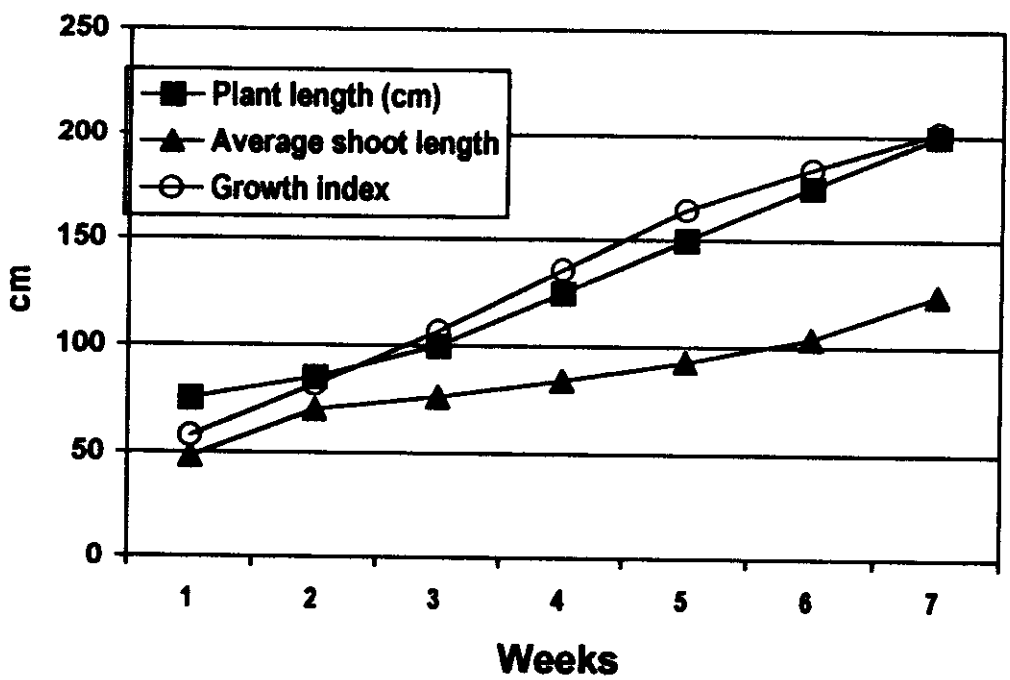
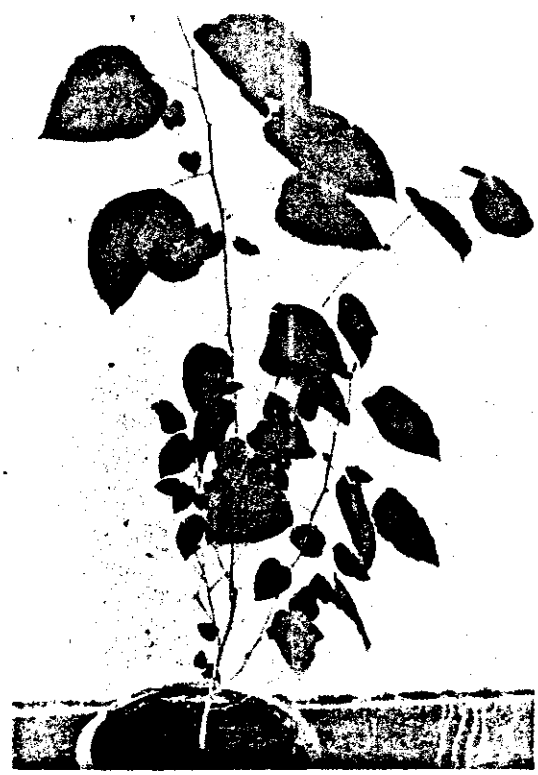


Fig. (18): First adaptation phase curve (Laboratory phase).

Laboratory phase

Laboratory phase



Greenhouse phase

Field phase

Photo (16): Different acclimatization phases of Mulberry plants.

IV.B.2. Greenhouse phase:

Moreover, the behavior of the adopted plants during the second stage of adaptation (greenhouse acclimatization) was recorded in **Table (16-b)** and plotted in curve, **Fig. (19).** This reflect that the aforementioned measured growth parameters increased as the plants become older up to the 6th week after which sharp increase occurred during the latest three weeks (7^{th}, 8^{th}, and 9^{th} weeks).

Table (16-b): Growth development of acclimatized Mulberry during pre-transference to the greenhouse.

Reading Date of	Plant length (cm)	Number of shoots	Average shoot length	Number of green leaves	Growth index
Week (1)	5.0	4.0	3.0	7.0	4.75
Week (2)	6.5	4.0	3.25	10.0	5.94
Week (3)	8.0	5.0	4.0	13.0	7.50
Week (4)	10.0	6.0	5.5	17.0	9.60
Week (5)	12.0	7.0	7.0	21.0	11.88
Week (6)	16.0	8.0	10.0	25.0	14.75

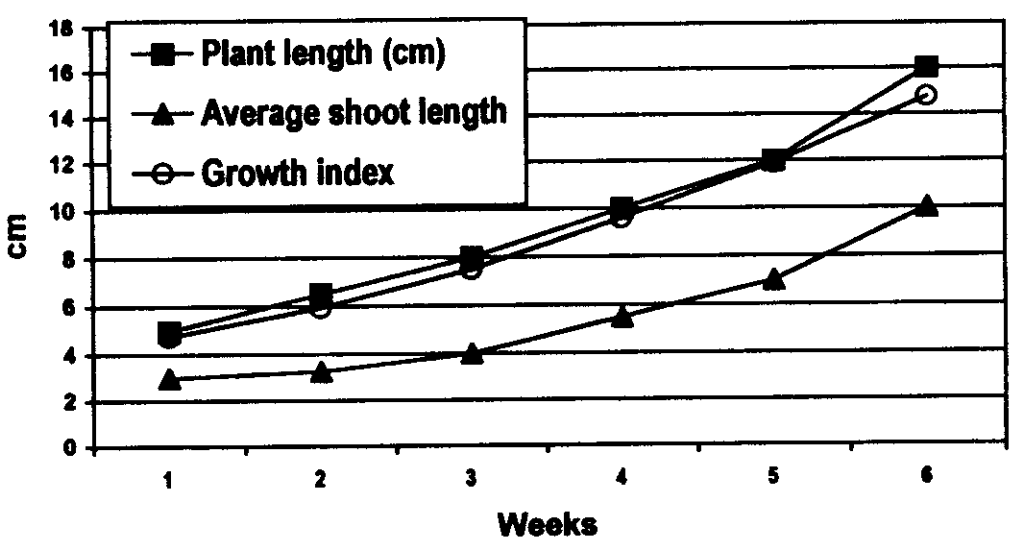


Fig. (19): Second adaptation phase curve (Greenhouse phase).

IV.B.3. Filed phase:

Furthermore, **Table (16-c), Fig. (20) and Photo (16)** clarify that studied growth parameters of the adapted plants in the third stage (permanent field) showed a noticeable increase during the first four weeks followed by a sharp increase during the rest reading periods.

Regarding the rate of increase in growth parameters during the three stages of gradual adaptation (laboratory, greenhouse and permanent field acclimatization) it is noted that the most rapid increase occurred during the third adaptation stage (permanent field) followed with the second adaptation stage (greenhouse acclimatization) and finally the laboratory acclimatization. This results may be due to the amount of the formed root system during the third adaptation stage which encouraged an increase in the adsorption ability and in turn enhanced the growth parameters.

Table (16-c): Field phase:

Reading Date of	Plant length (cm)	Number of shoots	Average shoot length	Number of green leaves	Growth index
Week (1)	16	8	10.0	25	14.75
Week (2)	20	8	12.5	30	17.63
Week (3)	23	9	13.5	35	20.13
Week (4)	28	10	15.0	41	23.50
Week (5)	33	12	17.5	48	27.63
Week (6)	38	13	21.0	58	32.50
Week (7)	52	14	26.0	66	39.50
Week (8)	69	15	44.0	74	50.50
Week (9)	75	17	48.0	91	57.75

The aforementioned results indicate the success of acclimatization stage. These assured by the findings of **Hossain *et al.* (1992).** They indicated that the regenerated plantlets were successfully established in soil under filed conditions after a few days of indoor acclimatization.

Photo (17, A & B) deals with the different developmental phases of Mulberry plants.

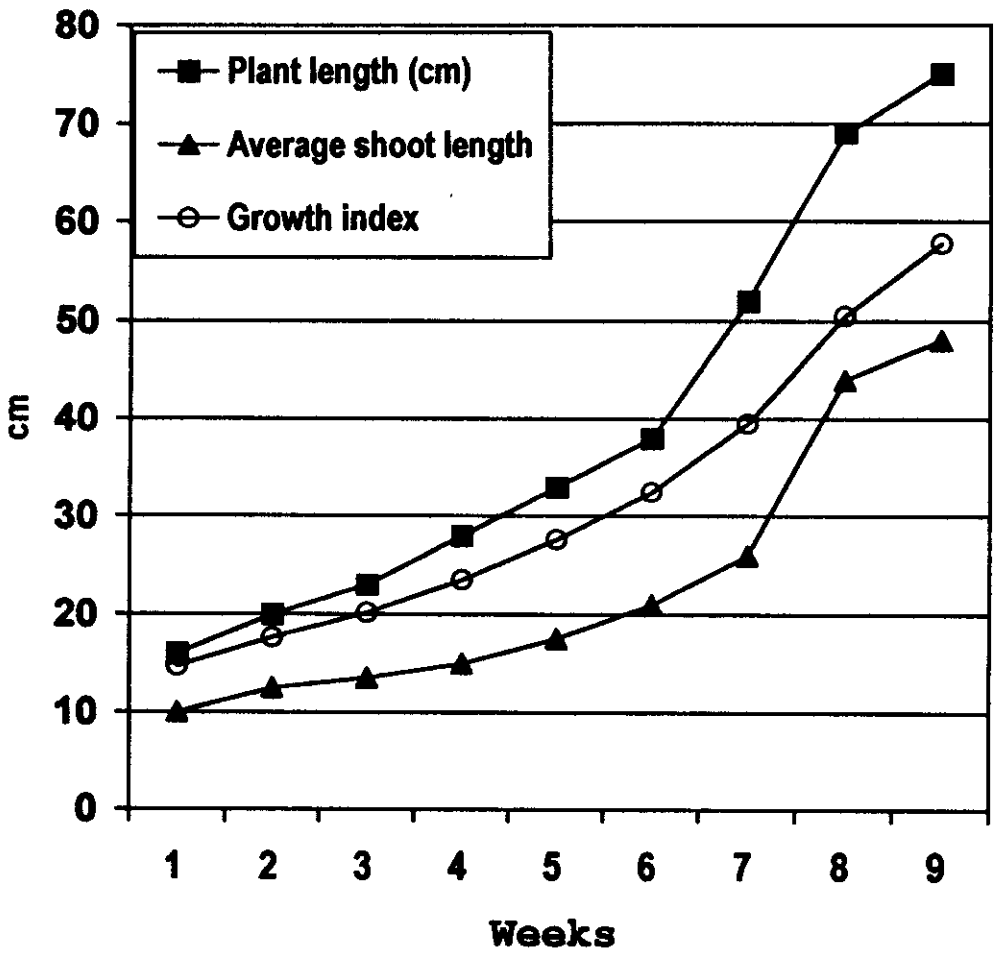


Fig. (20): Third adaptation phase curve.

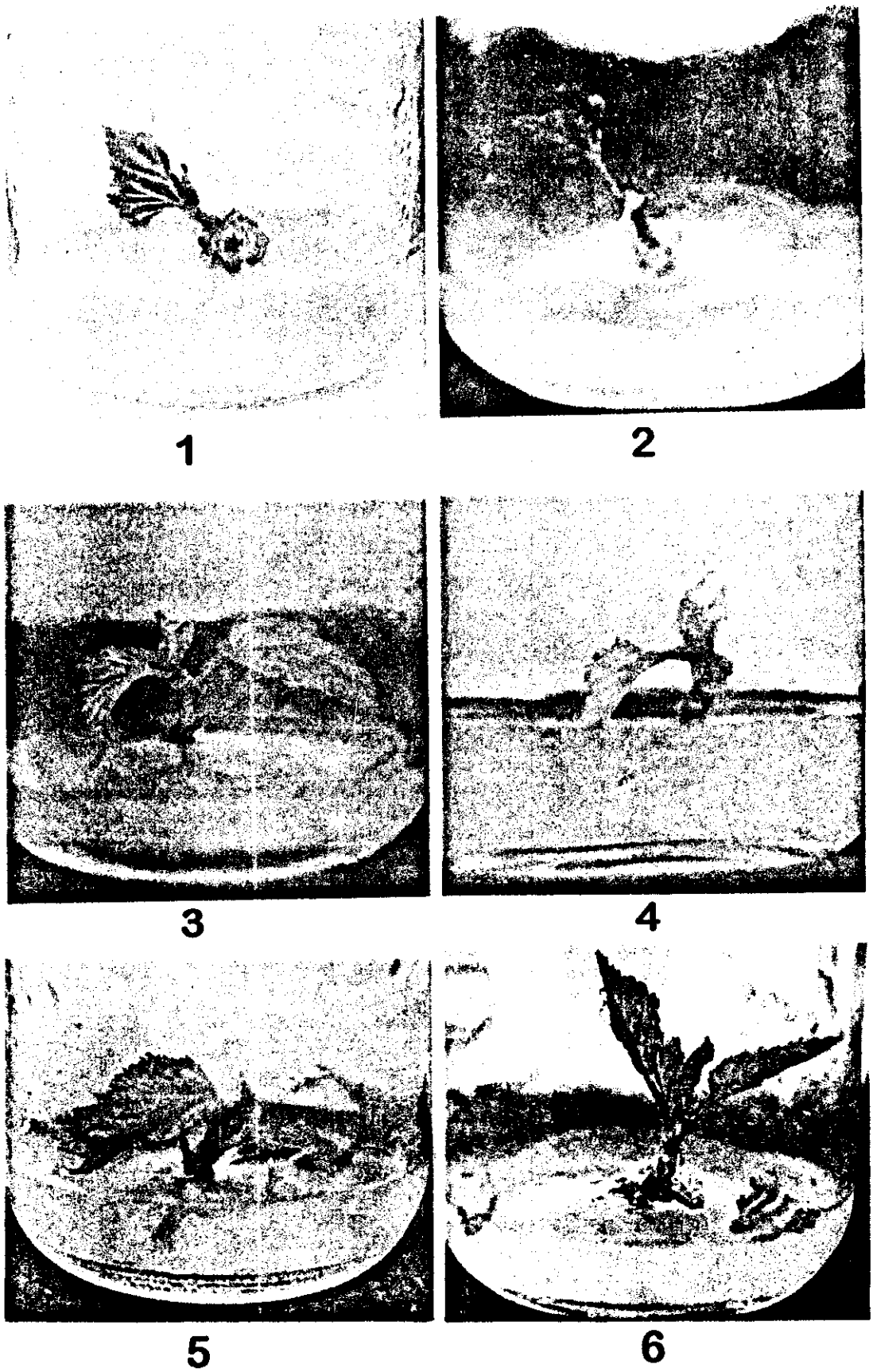
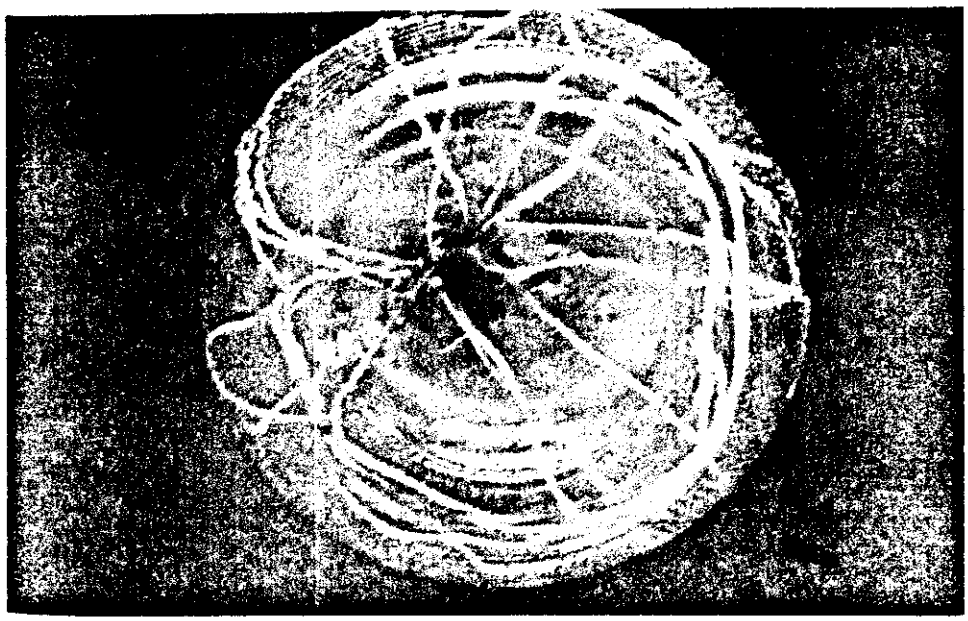


Photo (15-A): Developmental stages of Mulberry explants.



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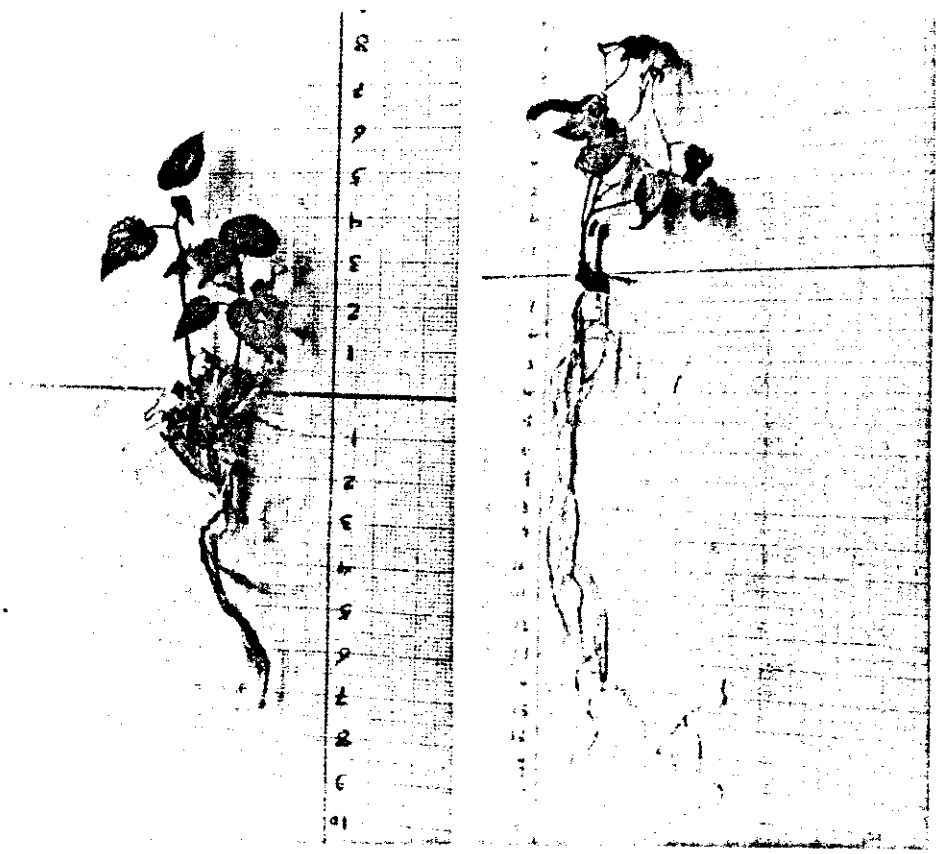

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Photo (15-B): Developmental stages of Mulberry explants.