Using of tissue culture to decrease the deterioration of productivity of *Pelargonium graveolens* plant in Egypt

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Abstract: *Pelargonium* plants are infected with different soil-borne fungi causing root rot and wilt diseases. This investigation aimed to production of pelargonium plants free from root rot and wilt diseases using tissue culture. Root rot and /or wilt fungal diseases were always detected in fields of ten districts of Beni-Suief, Minia and Fayoum governorates during 2008/2009 and 2009/2010 seasons. Mean percentages of infection on (2, 4,7&12 months old) during the two seasons ranged between (25.3,23.7,28.6,30.7,27.1%) respectively. During the experimental course of isolation, infected cuttings and plants yield six identified fungi, i.e. *Fusarium moniliforme*, *F. oxysporum, F. semitectum, F. solani*, *Macrophomina phaseolina* and *Rhizoctonia solani*. *F. oxysporum* (21.62%) and *F. moniliforme* (19.31%), however, were the most frequently fungi isolated from infected cuttings, followed by *F. semitectum* (15.44%) and *M. phaseolina* (12.74%). Whereas, *F. oxysporum* (21.50%) and *R. solani* (21.17%) gave the highest occurrence (%) from root rotted and or wilted plants, followed by *F. semitectum* (18.89%) and *F. moniliforme* (17.92%). In contrast, *F. solani*. (11.58%), *M. phaseolina* (9.77%) and *F. solani* (10.75) were isolated at low frequencies plants, respectively. The results of pathogenicity tests are as follows: Sowing cuttings in infested soil proved that all the isolated fungi from root rotted and /or wilted Pelargonium plants were pathogenic to cuttings, except *M. phaseolina*. *F. oxysporum*, *F. moniliforme* and *R. solani*, however, were the most virulent fungi, recorded the highest percentages of root rot and /or wilted.While, *M. phaseolina*, *F. solani, F. moniliforme* gave the low frequencies. On the other hand, the foliage growth parameters (no. of branches, fresh weight/ gm and no. of leaves) minimized by all pathogenic fungi. However, *F. oxysporum* and *R. solani* significantly realized the highest reductions, followed by *F. semitectum* and *F. moniliforme*. Planting fresh terminal cuttings in infested soil confirmed that all fungi tested were pathogenic and *F. oxysporum* (59.25%) and *F. semitectum* (53%) significantly recorded the highest infection (%), followed by *F. moniliforme* (40.50%). The superiority of these fungi was also found in decreasing foliage growth parameters, i.e. (no. of branches, fresh weight /gm and no. of leaves). *F. moniliforme* and *F. semitectum*, however, were significantly the most virulent fungi against foliage growth parameters in most cases. In contrast, *M. phaseolina* recorded the least reductions. Planting rooted terminal cuttings in infested soil confirmed that oil fungi tested were pathogenic and *F. oxysporum* (53%) and *R. solani* (46.75) significantly recorded the highest infection (%), followed by *F. semitectum* (40.50%), *F. moniliforme* (34.25%). The superiority of these fungi were also found in decreasing foliage growth parameters, i.e. (No. of branches, fresh weight (gm) and No. of Leaves). *F. oxysporum* and *R. solani* however, were significantly the most virulent fungi against foliage growth parameters in most case. In contrast *M. phaseolina* recorded the least reductions. Field experiments: tissue culture was significantly the most active normal cuttings. Nitrobien (5.88%), however, proved to be the superior treatment significantly, followed by Vitavax. These treatments led to decrease the infection percentage and increase the different measurements (No. of branches, Fresh weight, dry weight and oil percentage).
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1. Introduction

Medicinal and aromatic plants (approxately 60.000 feddans / year) have a great economical importance as they occupy an export priority in the first rank. They have a special importance among the other traditional crops in the Middle and Upper Egypt, especially in Giza, Fayoum, Beni- Suief, Minia and Assiut governorates. Pelargonium (*Pelargonium graveolens* L.) is one of the most important aromatic crops in many parts of the world as well as in Egypt. It is used also as ornamental crop in many gardens. The incidence of the diseases attacking pelargonium especially root rot and wilt seemed to be increased in Egypt according to: (1) The continuous cultivation in the same soil areas for long period, i.e. crop rotation is partially or completely neglected, (2) Absence of high yielding disease resistant varieties, (3) Lack of proper development or technology for growing and harvesting and (4) Lack of researches concerning present status and practical strategies for disease management. The areas cultivated with pelargonium

in Beni–suief Governorate was decreased from 5413 in 2004 to 2451 feddan in 2006, in Fayoum Governorate was decreased from 333 in 1989 to 17 feddan in 2004 and in Egypt was decreased from 5430 in 2004 to 2502 feddan in 2006 respectively(Dept. Agric.and Economical Static., Ministry of Agriculture).

The present study aimed to throw light on the importance of using tissue culture in decreasing the deterioration of productivity of *Pelargonium graveolens* plant in Egypt.

2. Materials and Methods

2.1. Disease survey

Percentages of naturally infected plants of Pelargonium (2, 4, 7, 12 months-old) by root rot and /or wilt diseases were recorded during 2008 /2009 and 2009 / 2010 seasons in Beni–Suief (Naser, Elwasta, Ehnasea districts & Beni-Suief) Fayoum (Fayoum, Ebshaway & Tamia), and Minia (Minia, Samalot & El-Adoa districts) governorates. External symptoms, however, are consisting of withering on one branch or more, yellowing, stunting, wilting and basal stem rot. Samples from infected plants were collected for isolation trials in laboratory.

2.2. Isolation, purification and identification of the causal organisms

Pelargonium infected by root rot and /or wilt were collected from fields and nurseries of Beni-Suief, Fayoum and Minia governorates during 2008 /2009 and 2009/2010 seasons. Infected roots and basal stems were cut into small pieces, washed thoroughly with tap water, then surface sterilized with 3% sodium hypochlorite solution for 3 minutes, rinsed several times in sterilized distilled water and dried between sterilized filter papers. The pieces were plated on potato dextrose agar (PDA) medium in petri dishes, and incubated at 27^{O}C for 7 days with observation every day. The growing fungal colonies were microscopically examined, counted and the frequency of each fungus was calcuated. Purification of the isolated fungi was carried out using single spore or hyphal tip techniques suggested by Dhingra and Sinclair (1995). These fungi, however, were identified according to Barnett and Hunter (1960), Booth (1977), Domsch *et al.* (1980) and Plaats–Niterink (1981). The identification, however, was kindly comfirmed by Mycology and Plant Diseases Survey Dep., Pl.Pathol.Res.Inst., Agric.Res.Center, Giza, Egypt. Pure cultures grown onto PDA slants were kept at low temperature (5^{O}C) for further studies.

2.3. Pathogenicity studies

Pathogenicity experiments were carried out according to Halawa, (2004).

2.4. Production of root rot and wilt-free *Pelargonium* plants using tissue culture

Among the prevalent tissue culture techniques of agricultural and horticultural promise, meristem, shoot tip culture and nodal cuttings have exploited at a much wider scale primarily due to their application in diverse areas such as rapid clonal multiplication of vegetatively propagated. Crop plans, virus elimination and germplasm preservation of both vegetatively and seed propagated crops (Nehra and Kartha 1994 and Faccidi and Marani, 1998) and more recently in the development genetic transformation protocols for crop improvement through gene transfer.

2.4.1. Culture media

Media used in this investigation was salt mix of Murashige and Skoog's medium (1962) “Sigma”, 4.4 g/L Table (1) consentient required in large quantities e.g. sucrose 30 g/L and agar 7 g/L were weighted at the time of medium preparation. Hormones required depend on the stage carried on as in Table (1). Chemicals were dissolved into a liter of distilled water and the pH was adjusted to 5.8. Agar at the rate of 7 g/L was added to medium. Medium was then dispensed into 250 ml culture jars containing 30 ml medium, then autoclaved at 121°C under pressure of 1.2 Ib/inch/cm^2 for 20 minutes.

Table 1. Ingredients of MS medium salts and vitamins solutions

Constituents	Starting	Shooting	Rooting
MS salt g/L.	4.4	4.4	4.4
Sucrose g/L.	30	30	30
BAP mg/L.	0.25	1.0	-
NAA mg/L.	-	0.2	0.4
Agar g/L.	7	7	7

2.4.2. Sterilization method

Shoot tips (10cm-long) from new branches of *Pelargonium* cultivars were collected. All foliage leaves were excluded and short segments of each leaf petiole were left attached to the stem covering the axillary bud. The segments were washed in running tap water for 30 min. Service sterilization for 20 min in Clorox (commercial solution of sodium hypochlorite, 5.25 active ingredients) at 25%. After sterilization the explants were rinsed third in sterilized water.

2.4.3. Micropropagation of stock plants (rot rot and wilt-infected plants)

The infected explants were aseptically cut into single nodal pieces each with small segments of leaf petiole that covered the axillary bud, some ends of the cuttings were recut to remove areas which damaged by bleach treatment. The base of each cutting was cultured in jars contained 25ml of MS medium described before supplemented with 0.25 ml/L BA and kept at 25°C under florescent light. After generation to plantlets, shoots were used for propagation in MS-medium plants were maintained by propagation nodal cuttings on MS-medium supplemented with vitamins according to Roest and Bokelmann (1975), 2% (w/v) sucrose, 0.5mg/L BA for 16 h photoperiod provided by 2500-3000 lux cool white fluorescent lamps, plantlets became available for sub culturing or for therapeutic operation.

2.4.4. *In vitro* propagation of treated shoots

Shoots that grew *in vitro* from the buds that cultured on free hormone media and after therapeutic operations were cut into single node segments with some leaves and inserted in media containing 0.5mg/L BA.

2.4.5. Rooting stage

To induce roots, the microprpagatrd were transferred to media containing NAA at 0.4 mg/L. After 21 days plants were observed.

3. Results

3.1. Disease survey

Percentages of natural infected plants showing disease syndrome, i.e. withering, discoloration or yellowing, stunting, wilting, rotted roots or basal stem rot occurred on those growing under nurser and field conditions of ten districts of Beni-Suief, Minia and Fayoum Governorates during 2008/2009 and 2009/2010 season were recorded Table (2) Root rot and wilt diseases were always found in all plantations exaimend in the surveyed governorate districts. Mean percentages of infection in Beni-Suief recorded 25.3 infection percent. On the other hand, infection percentages were always increased in both surveyed seasons by the increasing in plant age. They were (22.3 & 27%) for 2-month-old plants in both surveyed years increased to (30 & 29.8%) for those of 12- month-old grown in Beni-Suief districts.The highest mean percentages of infection for survey season and plant ages tested were recorded in Naser (31.5%) and Beni-Suief (25.5%) districts(Beni-Suief Governorate), Beba (34.3%) and Ehnasea (30.4%) districts(Beni-Suief) and Samalot district (33.7%) in Minia. In contrast, the least mean of infection in the three surveyed Governorates during 2008/2009 and 2009/2010 seasons, mean percentages of infection on both plant ages (2&4 months-old) ranged between (25.9 -29.7%).

3.2. Isolation, purification and identification of the causal fungal organisms and their frequencies

Isolation trials were carried out from naturally infected Pelargonium plants showing root rot and /or wilt symptoms from different localities in Beni-Suief, Minia and Fayoum Governorates. During the experimental course of isolation fungi were isolated and identified according to their morphological characters.

Table (3) *F.oxysporum* (21.62 %) and *Fusarium moniliforme* (19.31 %) were the most frequently fungi isolated from normal cuttings, followed by *Rhizoctonia solani* (19.31%) and, *F. semitectum*, (15.44%). Whereas, *F. solani* (12.74%) and *Macrophomina phaseolina* (11.58 %) were isolated at low frequencies. On the other hand, *F. oxysporum* (21.50 %) and *R. solani* (21.17%) gave the highest occurrence (%) from root rotted plants, followed by *F. semitectum* (18.89%) and *Fusarium moniliforme* (17.92%). In contrast, *F. solani* (10.75%) and *M. phaseolina* (9.77%) were infrequently associating fungi to rotted roots.

3.3. Pathogenicity tests

3.3.1. Sowing fresh terminal cuttings in infested soil

A. Infection (%)

Data in Table (4) indicate that all fungi tested were pathogenic to Pelargonium plants as they significantly increased percentages of Root Rot and wilt compared with the control treatment (non infested soil*) F. oxysporum* (59.25 %), *F. semitectum* (53.00%*)* and *R. solani* (53%) recorded the highest percentages of Root Rot and wilt, respectively. In contrast, *F. solani* and *M. phaseolina*, were the least effective fungi in this respect. On the other hand*, F. oxysporum* resulted in the highest significant percentage of wilt compared with the other fungi.

B. Growth parameters for plants developed from fresh terminal cuttings

Data presented in Table (4) reveal that all tested fungi significantly minimized Pelargonium cuttings (no. of branches, fresh weight and no. of leaves) compared with those of control treatment, *F. moniliforme* and *F. semitectum* realized the highest reduction as for all the previous parameters with significant differences compared with the other fungi tested, In general, *F. moniliforme* recorded no. of branches (3.4), (14.5) no. of leaves and fresh weight

(142.5gm) compared with (12.23, (45) and 335gm with plants of control treatment.

Table 2. Percentages of natural infection by root rot and wilt fungal disease on pelargonium plants grown in three Governorates during 2008/2009 and 2009/2010 seasons.

Governorates	2008/2009					2009/2010					Mean
	2months	4 months	7 months	12 months	mean	2 months	4 months	7 months	12 months	mean	
Beni-suief											
Naser	20.8	32.7	35.6	36.0	31.3	25.5	27.5	36.4	37.5	31.7	31.5
Elwasta	26.4	9.8	20.0	25.0	20.3	30.9	10.5	24.4	25.7	22.9	21.6
Ehnasea	19.7	14.9	25.6	28.9	22.3	25.0	13.8	23.9	29.4	23	22.7
Beni-suief	22.4	23.7	28.7	30.2	26.3	26.7	20.5	25.6	26.5	24.8	25.6
mean	22.3	20.3	27.5	30	25	27	18	27.6	29.8	25.6	25.4
Fayoum											
Fayoum	28.1	30.7	36.5	38.7	33.5	30	25.4	34.4	35.6	31.4	32.6
Ebshaway	25.4	10.9	15.9	20.3	18.1	28.6	11.0	14.8	16.8	17.8	18
Tamea	20.8	25.8	30.0	33.4	27.5	25.9	25.8	30.0	33.5	28.8	28.2
mean	24.8	22.5	27.5	30.8	26.4	28.2	20.7	26.4	28.6	26	26.3
Minia											
Minia	22.8	33.9	36.3	37.4	32.6	25.7	30.8	34.4	35.6	31.6	32.1
Samalot	25.5	20.6	27.6	27.9	25.4	29.5	23.3	25.7	26.7	26.3	25.9
ElAdoa	20.8	24.4	32.9	34.4	28.1	23.9	24.5	30.0	33.0	27.9	28
mean	23.00	26.3	32.3	33.2	29	26.4	26.2	30	31.8	28.6	28.7
Mean	23.4	23	29.1	31.3	26.8	27.2	21.6	28	30.0	26.7	26.7

Table 3. Frequency percentages of the fungi isolated from damped seedlings and root rotted Pelargonium plants.

Fungi	Normal cuttings (45-day –old)		Root rotted and/or wilted plants.	
	Isolates No.	Frequency (%)	Isolates No.	Frequency (%)
Fusarium moniliforme J.Sheld.	50	19.31	55	17.92
F.oxysporium Schlecht.	56	21.62	66	21.50
F.semitectum Berk. & Rav.	40	15.44	58	18.89
F.solani (Mart.) Sacc.	33	12.74	33	10.75
Macrophomina phaseolina (Tassi) Goid	30	11.58	30	9.77
Rhizoctonia solani Kühn	50	19.3	65	21.17
Total	259	100.00	307	100.00

Table 4. Percentages of infection of root root rot and wilt diseases, number of branches, fresh weight (gm), number of leaves from *Pelargonium graveolens* plants (fresh terminal cuttings) infested soil, under greenhouse conditions.

Fungi	Infection % root rot and wilt	No. of branches	Fresh weight	No. of leaves
F. moniliform	40.50	3.40	142.50	14.50
F. oxysporum	59.25	5.28	185.00	23.25
F. semitectum	53.00	4.18	172.50	22.75
F. solani	34.25	6.03	127.50	25.75
M. phaseolina	15.50	8.28	97.25	24.75
R. solani	5.300	7.13	180.00	36.25
Control	0.00	12.23	335.00	45.00
L.S.D at 5%	9.493	0.188	22.33	6.731

3.3.2. Sowing rotted terminal cuttings

A. Infection (%)

Data in Table (5) indicate that all fungi tested were pathogenic to Pelargonium plants as they significantly increased percentages of Root rot and wilt compares with the control treatment (non infestd soil). *F. oxysporum* (53%) and *R. solani* (46.75%) recorded the highest percentages of root rot and / or wilt respectively. In contrast, *F. solani* (28%) and *M. phaseolina* (10.75%) were the least effective fungi in this respect. On the other hand, *F. oxysporum* resulted in the highest significant percentage of wilt compared with the other fungi.

B. Growth parameters for plants developed from rotted terminal cuttings

Data presented in Table (5) revealed that, all tested fungi significantly minimized Pelargonium plants no. of branches, fresh weight (g) and no. of leaves compared with those of control treatment, *R. solani*, *F.oxysporum* and *Fusarium moniliforme*, realized the highest reduction as for all the previous parameters with significant differences compared with the other fungi tested. In general, *R.solani* recorded no. of branches (6.10%), (27%) no. of leaves and fresh weight (57.50gm) compared with 13.35, 75.75 and 335gm with plants of control treatments.

3.4. Laboratory experiments

3.4.1. Production of root rot and wilt-free *Pelargonium* plants using tissue culture

For establishment stage, infected shoot tips (explants) were cultivated after sterilization process in media containing 0.5mg/L BAP that mainly resulted in the growth of stem, leaves and occurrence of auxiliary shoots. While the micropropagated stage was done by sub cutting of the stem cuttings up to two times every 21 days Fig (1).

3.5. Field experiment

3.5.1. Infection

Data presented in Table (6) revealed that, plants resulted from tissue culture was significantly the most active plants resulted from normal cuttings during 2008/2009 and 2009/2010. Mean percentages of infection plants resulted from tissue culture (17.07%) in 2008/2009 and (15.35%) in 2009/2010, while mean percentages of infection on plants resulted from normal cuttings (22.42%) in 2008/2009, (25.32%) in 2009/2010. Plant Guard (9.88%), however, proved to be the superior treatment significantly, followed by Rhizo-N (10.13%) in 2008/2009, Plant Guard (11.00%), however, proved to be the superior treatment significantly, in 2009/2010.

3.5.2. Number of branches

Data presented in Table (7) revealed that, plants resulted from tissue culture was significantly the most active plants resulted from normal cuttings during 2008/2009 and 2009/2010. Number of branches of plants resulted from tissue culture (36.92%) in 2008/2009 and (36.50%) in 2009/2010, while Number of branches of plants resulted from normal cuttings (34.64%) in 2008/2009, (35.50%) in 2009/2010. Plant Guard (53.88 branch), however, proved to be the superior treatment significantly, followed by Rhizo-N (52.50 branch) in 2008/2009, Rhizo-N (52.50 branch), however, proved to be the superior treatment significantly, in 2009/2010.

3.5.3. Fresh weight

Data presented in Table (8) revealed that, plants resulted from tissue culture was significantly the most active plants resulted from normal cuttings during 2008/2009 and 2009/2010. Fresh weight of plants resulted from tissue culture (3187.03 gm) in 2008/2009 and (3236.25 gm) in 2009/2010, while fresh weight of plants resulted from normal cuttings (2412.18 gm) in 2008/2009, (2397.07 gm) in 2009/2010. Plant Guard (3494.62 gm), however, proved to be the superior treatment significantly, followed by Rhizo-N (3426.25gm) in 2008/2009, Potasein (3510.25gm), however, proved to be the superior treatment significantly, in 2009/2010.

3.5.4. Dry weight

Data presented in Table (9) revealed that, plants resulted from tissue culture was significantly the most active plants resulted from normal cuttings during 2008/2009 and 2009/2010. Dry weight of plants resulted from tissue culture (277.55 gm) in 2008/2009 and (284.08 gm) in 2009/2010, while dry weight of plants resulted from normal cuttings (198.25 gm) in 2008/2009, (175.89 gm) in 2009/2010. Plant Guard (309.25gm), however, proved to be the superior treatment significantly, followed by Rhizo-N (302.88 gm) in 2008/2009, Plant Guard (297.13gm), however, proved to be the superior treatment significantly, in 2009/2010.

3.5.5. Oil content

Data presented in **Table (10)** revealed that, plants resulted from tissue culture was significantly the most active plants resulted from normal cuttings during 2008/2009 and 2009/2010. oil content of plants resulted from tissue culture (0.390%) in 2008/2009 and (0.312%) in 2009/2010, while oil content of plants resulted from normal cuttings (0.207%) in 2008/2009, (0.213%) in 2009/2010. Plant Guard (0.336%), however, proved to be the superior treatment significantly, followed by Rhizo-N

(0.433%) in 2008/2009, Rhizo-N (0.466%), however, proved to be the superior treatment significantly, in 2009/2010.

Table 5. Percentages of infection of root root rot and wilt diseases, number of branches, fresh weight (gm), number of leaves and from *Pelargonium graveolens* L. plants (rooted terminal cuttings) in infested soil, under greenhouse conditions.

Fungi	Infection% root rot and wilt	No. of branches	Fresh weight	No. of leaves
F. moniliform	34.25	8.150	83.75	41.25
F. oxysporum	53.00	6.00	94.25	31.25
F. semitectum	40.50	8.20	87.25	39.50
F. solani	28.00	11.30	75.75	52.75
M. phaseolina	10.75	11.88	94	57.50
R. solani	46.75	6.10	57.50	27.00
Control	0.00	13.35	335.00	75.75
L.S.D.at 5%	6.572	1.582	18.76	7.977

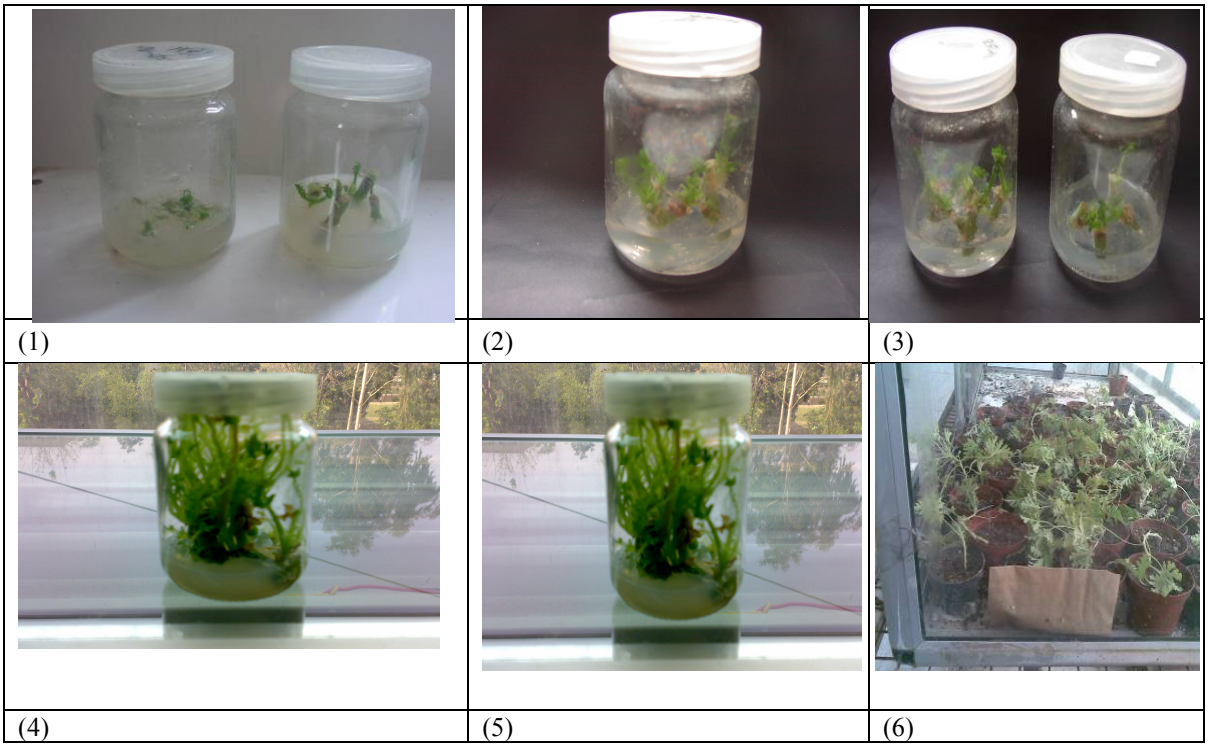

Figure 1. Different stages of using tissue culture for production of root rot and wilt-free pelargonium plants.

Table 6. Effect of various control measures on infection root rot and wilt diseases *Pelargonium graveolans* plants, grown under naturally infected field soil, during 2008/2009 and 2009/2010 seasons resulting from normal cuttings and tissue culture transplants.

Treatments	2008/2009			2009/2010		
	Type of culture			Type of culture		
	Tissue culture	Normal cutting	Mean	Tissue culture	Normal cutting	Mean
Bioproductes						
Plant Guard	5.50	14.25	9.88	5.00	17.00	11.00
Rizo –N	7.25	13.00	10.13	6.75	15.75	11.25
Plant extract						
Marjoram	15.25	16.50	15.88	13.50	35.00	24.25
Thyme	16.50	18.25	17.38	14.25	18.25	16.25
Other com.						
Ascopein	10.50	15.00	12.75	8.00	16.25	12.13
Potasein	12.00	27.00	19.53	9.25	25.00	17.13
Control	52.50	53.00	52.75	50.75	50.00	50.38
Mean	17.07	22.42		15.35	25.32	

L.S.D. at 5% for: Agriculture type (A) = 3.1, Agriculture type (A) = 4.25, Treatments (T) = 3.47, Treatments (T) = 6.51
A X T = 4.89, A X T = 9.2

Table 7. Effect of various control measures on number of branches of *Pelargonium graveolans* plants, grown under naturally infected field soil, during 2008/2009 and 2009/2010 seasons resulting from normal cuttings and tissue culture transplants.

Treatments	2008/2009			2009/2010		
	Type of culture			Type of culture		
	Tissue culture	Normal cutting	Mean	Tissue culture	Normal cutting	Mean
Bioproductes						
Plant Guard	53.50	54.25	53.88	54.00	55.50	54.75
Rizo –N	54.00	51.00	52.50	53.00	53.00	53.00
Plant extract						
Marjoram	16.50	16.50	16.50	15.25	15.50	15.37
Thyme	44.75	42.25	43.50	44.25	42.50	43.38
Other com.						
Ascopein	37.50	36.50	37.00	39.00	37.00	38.00
Potasein	27.25	23.75	25.50	25.75	23.50	24.62
Control	25.00	18.25	21.62	24.25	21.50	22.87
Mean	36.92	34.64		36.50	35.50	

L.S.D. at 5% for: Agriculture type (A) = 1.5, Agriculture type (A) = N.S., Treatments (T) = 2.7, Treatments (T) = 2.8
A X T = 3.8, A X T = 4.0

Table 8. Effect of various control measures on fresh weight (gm) of *Pelargonium graveolans* plants, grown under naturally infected field soil, during 2008/2009 and 2009/2010 seasons resulting from normal cuttings and tissue culture transplants.

Treatments	2008/2009			2009/2010		
	Type of culture			Type of culture		
	Tissue culture	Normal cutting	Mean	Tissue culture	Normal cutting	Mean
Bioproductes						
Plant Guard	3909.25	3080.00	3494.62	3406.25	3025.00	3215.62
Rizo –N	3875.00	2977.50	3426.25	3705.00	2064.00	2884.50
Plant extract						
Marjoram	3711.25	3045.00	3378.12	3610.00	2044.25	2827.12
Thyme	3615.00	2520.00	3067.50	3845.00	3110.50	3477.75
Other com.						
Ascopein	3848.75	2565.00	3206.87	3835.00	3087.50	3461.25
Potasein	3025.00	2352.75	2688.87	3906.25	3114.25	3510.25
Control	325.00	345.00	335.00	346.25	325.00	335.63
Mean	3187.03	2412.18	19597.23	3236.25	2397.07	42756.71

L.S.D. at 5% for: Agriculture type (A) = 73.17, Agriculture type (A) = 54.39, Treatments (T) = 97.22, Treatments (T) = 115.6

A X T = 137.50, A X T = 163.5

Table 9. Effect of various control measures on dry weight (gm) of *Pelargonium graveolans* plants, grown under naturally infected field soil, during 2008/2009 and 2009/2010 seasons resulting from normal cuttings and tissue culture transplants.

Treatments	2008/2009			2009/2010		
	Type of culture			Type of culture		
	Tissue culture	Normal cutting	Mean	Tissue culture	Normal cutting	Mean
Bioproductes						
Plant Guard	352.25	266.25	309.25	354.25	240.00	297.13
Rizo –N	350.70	255.00	302.88	351.25	225.00	288.13
Plant extract						
Marjoram	265.00	147.25	206.138	265.00	146.25	205.63
Thyme	335.75	246.00	290.88	336.25	208.75	272.50
Other com.						
Ascopein	313.25	228.50	270.88	316.25	194.25	255.25
Potasein	311.25	216.25	263.75	311.75	191.25	251.50
Smulleleits	259.00	196.00	245.50	300.00	178.75	239.38
Yeast	272.50	186.75	229.63	280.00	158.75	219.38
Control	38.25	42.25	40.25	42.00	40.00	41.00
Mean	277.55	198.25		284.08	175.89	

L.S.D. at 5% for: Agriculture type (A) = 13.15, Agriculture type (A) = 5.5, Treatments (T) = 18.08, Treatments (T) = 31.14

A X T = 25.58, A X T = 34.25

Table 10. Effect of various control measures on oil content of *Pelargonium graveolans* grown under naturally infected field soil, during 2008/2009 plants, and 2009/2010 seasons resulting from normal cuttings and tissue culture.

Treatments	2008/2009			2009/2010		
	Type of culture			Type of culture		
	Tissue culture	Normal cutting	Mean	Tissue culture	Normal cutting	Mean
Bioproductes						
Plant Guard	0.400	0.272	0.336	0.400	0.269	0.334
Rizo –N	0.602	0.264	0.433	0.670	0.262	0.466
Plant extract						
Marjoram	0.451	0.184	0.317	0.450	0.211	0.330
Thyme	0.301	0.174	0.237	0.320	0.189	0.254
Other com.						
Ascopein	0.351	0.244	0.272	0.320	0.236	0.278
Potasein	0.400	0.211	0.305	0.450	0.225	0.337
Control	0.230	0.101	0.165	0.200	0.100	0.150
Mean	0.3907	.20710		.31220	0.2131	

L.S.D. at 5% for: Agriculture type (A) = 0.070, Agriculture type (A) = 0.02, Treatments (T) = 0.100, Treatments (T) = 0.13

A X T = 0.21, A X T = 0.22

4. Discussion

Pelargonium (*Pelargonium gravulens* L.) is subjected to infection by several soilborne fungi, causing root rot and wilt diseases which represent a major problem in Egypt during the last decade as evident in survey trial seasons (2008/2009 &2009 &2010). Important and destructive diseases, however, were previously recorded on Pelargonium by Hilal (1985). On the other hand, mean of infection (%) on Pelargonium plants during seasons of disease survey were (25.4-26.3%) and (28.7%) (10 districts of Beni-Suief, Fayoum, and Minia Governorates), respectively.The highest diseases occurrence were in Fayoum (32.4%), Minia (32.4%) and Naser 31.5% districts of Fayoum, Minia Beni-Suief governorates,,respectively. The high diseases occurrence which was recorded on Pelargonium plants in some governorate districts might due to: (1) Absence the crop rotation in the cultivated fields, which lead to accumulate the fungal propagules in soil, (2) Absence the useful control programme against these diseases in nurseries and fields, (3) Absence of high yielding and disease resistant varieties and (4) Lack of researches concerning the present status and the future strategies for cultivation and diseases management. Isolation from root rotted or wilted plants yielded 259 and 307 fungal isolates respectively. They however, were identified to their species as *Fusarium moniliforme*, *F. oxysporum, F. semitectum, F. solani, Macrophomina phaseolina and Rhizoctonia solani*. In this respect, Hilal (1985) in Egypt isolated all these fungi, from Pelargonium exhibiting the same disease symptoms, except *F.moniliforme*.

On the other hand, *R. solani*, and *F. oxysporum* were the most frequently fungi isolated from cuttings, followed by *F. moniliforme* and *F. semitectum*.While, *F. oxysporum* and *R. solani*, followed by *F. semitectum* and *F. solani* recorded the highest frequencies (%) in root rotted and wilted Pelargonium plants. These results are in harmony with those reported by Hilal (1985), who mentioned that *Fusarium* spp. and *R. solani* gave the highest frequencies (%) in trials of isolation from wilted and root rotted Pelargonium plants. Pathogenicity tests were performed by planting fresh terminal cuttings in infested soil. The isolated fungi tested proved that they were pathogenic to cuttings, except *M. phaseolina* with cuttings. *F. oxysporum* and *F. semitectum* on root rot on plants. However, were the most virulent fungi in this respect? According to the available literature, *F. moniliforme F. oxysporum, F. solani* and *F. semitectum* were recorded for the first time in Egypt as causal pathogens of root rot diseases. The present results coincide with Hilal (1985), since he found that *F. semitectum* and *R. solani* were the most pathogenic fungi causing Root Rot and Wilt to Pelargonium plants. On the other hand, several medicinal and aromatic plants, not belong to *Geraniaceae* family, were found to be high susceptible to infect by soilborne fungi similar to those of Pelargonium. However, one fungus or more of *F. oxysporum, F. semitectum*, *F. solani* and *R. solani* is (or are) the most virulent pathogen (s)

responsible for root rot on senna (Abdelal *et al*. 1979 & Essmat *et al*. 1995), marjoram (Hilal *et al*. 1990), black–cumin (Hilal *et al*. 1994 b), roselle (Hilal *et al*. 1994a & Mahmoud, 2004), sweet basil (El-Shazly, 1996& Garibaldi *et al*. 1997), coriander (Mansour *et al*. 2000), stevia (Hilal and Baiuomy, 2000) and rue (Helmy *et al.* 2001). Also, one fungus or more from the above mentioned fungi is (or are) virulent pathogen(s) to marjoram plants (Hilal *et al*. 1990)and rue plants (Helmy *et al*. 2001). Growth parameters of Pelargonium plants were greately affected significantly as a result of infection by most of root rot and wilt pathogenic fungi tested. *F. moniliforme* and *F. semitectum* realized the highest significant reductions in foliage growth parameters of plants (no. of branches and fresh weight and no. of leaves. The present results are in accordance with Hilal (1985), who reported that plant height, no. of branches and fresh weight/ Pelargonium plants were significantly affected by *F. semitectum* and *R.solani* than the other pathogenic fungi. Also, the higher reduction in plant growth parameters of safflower (El-Deeb *et al*. 1983) and stevia (Hilal and Baiuomy, 2000) were recorded with infection by *(F. oxysporum* & *F. semitectum*) and (*F. oxysporum* & *R. solani*), respectively.

Production of pathogen-free pelargonium plantlets: when plants are infected with a pathogen, it is usually impossible to cure them. In practice, this may have less serious long-term consequences for annual or biennial crop plants, as a majority of pathogen is not seed-borne and their progeny may, therefore, be free from pathogen. Chronically infected perennials or vegetatively propagated crop plants, on the other hand, constitute a permanent source of infection. Meristem culture method has proved to be useful to get pathogen-free plants from infected stock material. Meristem culture was originally based on the assumption that, even in systematically infected plants, cells of meristematic tissues were not invaded by the pathogen, as in some cases a small zone near the shoot tip had been found to be free from pathogen. The technique developed involved culturing of supposedly pathogen -free cells and growing them into healthy plants. The meristem tip culture consists of excision of the meristem tip from the infected plants, its aseptic culture on a nutrient medium and transfer of the regenerated plantlet to the soil. Meristem culture is nowadays often followed by *in vitro* mass propagation of the pathogen-free plant. The first concern of the present work was to find out the best of the tissue culture techniques that support growth and development of potato explants and production of pathogen -free plantlets. Nodal cuttings procedure was used successfully for production of pathogen -free potato plantlets. These results were agreed with that obtained by Foxe *et al.* (1984); Melleor and Stace-Smith (1987); Facciolo *et al.* (1988); Aurkhes *et al.* (1991); Kayim and Koc (1992); and Ruzic *et al.* (1996). It could be concluded from the above mentioned results that the significance of the nodal cuttings technique is beneficial as a tool in increasing the efficiency of tissue culture techniques as a mean of getting virus free potato plants due to one or more of the following suggestions: 1.As plantlets are segmented into relatively large pieces, the chance of getting 100% growth of these segments into plantlets are quite high. 2. The amount of virus-free plant materials could be multiplied 5-7 folds using the nodal cutting method. 3. Nodal cutting method requires small space to maintain a large number of virus-free plants. 4. It certainly help the laboratories or the researcher to culture larger portions of nodes, thus insuring a higher degree of success in obtaining pathogen -free plantlets. 5. Finally, these materials can be used directly for production or as mother plants which can be used as a source of cuttings and thereby reducing virus concentration of fields allocated to the production of seed potato are free of pathogen, high yielding and acceptable to the growers.

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