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Effect of plant growth regulators, explant type on plantlet regeneration through callus induction in *Nerium oleander* L.

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ABSTRACT

It was to cultured leaf explant separated from the mature oleander's plant on MS media. Equipped with 2,4-D at 2mg/l concentration and Kin at 1mg/l concentration .which give the highest percentage of callus induction and fresh and dry weight, but this callus had failed to differentiate into branches when cultured in regeneration media equipped with BA and IAA in different concentration. When cultured stem separated from oleander seedlings. Had given the highest percentage rate of callus induction and fresh and dry weight, when cultured in MS media equipped with Kin and NAA at 1.5mg/l concentration for each. And resulted carryout in the cultured of this callus in regeneration medium equipped with BA and NAA at 2 and 0.5mg/l concentration respectively, to differentiation to the highest rate of the number of branches with this combination. Cultured of this branches in MS media was equipped with IAA at 2mg/l concentration had given the highest percentage of rooting, the number and length of the roots. Highest percentage of success acclimatization when cultured these plantlet in media, consisting one size of sand to one size of Peat moos.

Keywords: *Nerium oleander*, callus induction, regeneration and rooting, MS media

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INTRODUCTION

The *Nerium Oleander* of evergreen plants, which belonged to the *Apocynaceae* family, the original home of the plant from North Africa and the Middle East, and also found in southern Europe and south-west of Asia [1,2], was cultured this plant as Ornamental in areas Subtropical. Also this plant had ability to tolerate high salinity, drought and heat. Plant height reaches more than four meters and plant leaves narrow and sharp from the summit and ranging in length from 10-22cm, plant flowers pink or white color, the ranges flower diameter is about 5cm [3,4]. There were two Oleander Common are *Nerium oleander* and *Thevetia peraviana* [5], the flowers, leaves juice and bark of oleander plant are used in the treatment of cancerous ulcers, carcinoma and cancer [6]. The most toxic compound in the oleander plant was Oleandrin and Nerine, which was a cardiac glycoside. It is present in all parts of the plant, especially in the extraction as exists high concentration [7]. Tissue culture technology and plant cells become of modern and useful ways in the plant field. This technology came out from the research department to the practical application, and this technique hired in the rapid propagation of plants to obtain a large number of plants so at any time of the year and in less time within the sterile space. A large number of researchers studied micro propagation and callus induction from different explants of the oleander plant like seedlings, leaves and fruits. Where showed [8] that the cultured of leave explant in MS media had given good results in callus induction and suspension culture. And reach [9] the possibility of callus induction when cultured the leaf explant in MS media equipped with 2,4-D at 4.5mg/l and BAP at 2.5mg/l concentration, where this combination gave the highest percentage of callus induction. The addition of 2,4-D and BA to culture media for callus induction , gave significantly rate from explant of Oleander plant (node and phalanges).but cultured of leaves growing in this media was the most productive of callus induction and significant compared to other explants [10]. And when cultured stem and leaves of the plant oleander on MS media equipped with IAA the positive impact in the callus induction, at the end of the cutting area for both explant plant. This callus cultured in regeneration medium equipped with BAP and IAA, which lead to significant in stimulating the formation of branches within two weeks, when cultured in regeneration medium and put them in the incubator.

Could [11] of the callus induction when cultured oleander leaves in B5 media equipped with 2,4- D at 9.05 *µm*/l. Moreover, BA at 4.4*µm* /l concentration and callus, which induced from mature, leaves differentiation to the roots. while callus induced from juvenile leaves had given somatic embryogenesis, and when cultured in MS medium equipped with 1% sucrose had developed into plantlets and then was transferred to a potted for acclimatization in the field, explained . [12] cultured leaves of oleander plant in MS media , callus failed to differentiation to branches when cultured in regeneration medium but differentiation to the roots. In addition, won [13] plantlets when cultured the explant vegetable part nodal in MS medium, equipped with NAA at 0.1 mg/l and BA at 0.5 mg/l concentration, where to abled to obtain branches and roots directly. was to increase the concentration of NAA to 0.2mg/l led to increase the production of branches to 95% and reached branches lengths to 5 cm. and obtain [14] axillary Shoots when cultured shoot tips, from seedlings of plant oleander in Schenk Hildebrandt medium. Equipped with BA or TDZ as callus induction from culture leaves separated from seedlings and leaves from mature plant .the percentage of callus differentiated from seedling leaves to the branches 40% compared to 5% from differentiation of leaves callus from mature plant. Then rooting branches when cultured in MS media free of hormones, or equipped with IBA reached to 90%, were also able acclimatization of these plantlets and reached success rate of acclimatization to 95 - 100%.

MATERIALS AND METHODS

Preparing culture medium

Prepared MS culture media [15]where all the salts of organic and inorganic dissolved, then add sucrose weighing 30 gm/l. culture media supplied with plant growth regulators 2,4-D + Kin, and NAA+ Kin according to the required concentration (o,0.5,1,1.5 and 2) . amended the PH at 5.8, then agar was added to the culture media at a concentration 7g/l. then mix ingredient using hot plate magnetic stirrer for dissolved components of culture media. Culture media distribute in vials dimensions of 8 × 2.5cm by 10 ml / vial, and then sterilized by autoclave at 121 C^0 and pressure 1.04 kg/cm for 20 minutes.

Sterilization of seeds

Before the culturing oleander seeds, washed with running water for several times, then placed in a flask, contain ethyl alcohol concentration of 95% for a minute. Then washed several times with sterile distilled water, and transfer to dishes glass contain sodium hypochlorite solution at 2% concentration for 5 minutes, then washed several times by sterile distilled water to remove the effect of sterilization compound. Sterile seeds cultured on MS medium contain half strength without growth regulators, this process would conducted within the laminar airflow, and then the container of seeds planted, placed on the incubators at 25±1 Cº for three weeks to get seedlings sterile.

Sterilization the explants of oleander plant

Leaves and stems would separate from mature *Nerium oleander*, and washed several times, with running water for half an hour. Then explant flooded with alcohol ethyl at a concentration of 95% for half a minute, then washed with sterilizer distilled water for several times. then flooded the leaves and stems with a solution of Sodium hypochlorite at concentration 1% for five minutes, then washed several times with sterile distilled water to remove the effect of sterile compound ,this process had been done in laminar air flow.

Callus Induction

Seedlings explant separate (leaves, stems, roots, epicotyl and hypocotyl) and cultured on MS media equipped with 2,4-D + Kin and NAA + Kin at (0, 0.5, 1, 1.5, 2 mg/l) concentrations for the first media. And the same concentration for the second media and placed in the incubator at temperature 25±1 cº for a month in light and dark conditions. As well as explant (leaves and stems) separated from mature plants were planted in the same media and the same previous conditions for cultured explant from seedlings. All explant cultured at a rate of three explant / treatment repeated ten times.

Measuring fresh and dry weight of callus

We measured fresh weight for callus by the sensitive balance within laminar airflow the dry weight would measure by placing callus in an electric oven at a temperature of 70 c° for 48 hours and then measured dry weight of callus until stability of weight.

Regeneration medium

After callus induction is complete, were cultured in maintenance media for several times, after that transfer the callus to MS media equipped with plant growth BA at concentrations (0, 0.5, 1, 2, 3 mg / l) and NAA at concentrations (0, 0.5, 1 mg / l) and placed callus at incubator conditions for a month to get shoots.

Rooting medium

Vegetative branches formed were separated and removed the remnants of callus, then cultured on MS medium equipped with IAA at concentration (0, 0.5, 1, 2, 3 mg / l), at a rate of 3 branches /treatment for the purpose of rooting, and placed in the incubator conditions for three weeks.

Statistical analysis

Carried out experiments using (R C B D) Randomized Complete Block Design and the results would analyzed and compared using Duncan test at the level 0.05 of probability [16].

RESULTS AND DISCUSSION

Choose the culture media and explant for callus induction

All the explant separated from the seedling (stem, leaves, roots, hypocotyl and epicotyl) failed in Callus induction, when cultured in MS media was equipped with 2,4- D + Kin for all concentrations in conditions of light and dark. While callus induced from leaves and stem separated from seedling when

cultured on MS media, was equipped with NAA +Kin in light conditions, while the rest of the explants (roots, hypocotyl and epicotyl) failed to Callus induction at light and dark condition, so it was excluded in subsequent experiments.

The combination of NAA + Kin may show different responses for Callus induction of both stem and leaves, which cultured under light conditions, 16 hours and lighting intensity 1000 Lux, where the percentage of Callus induction of leaves very few in most combinations of NAA and Kin, and to take a long time for induced. The quality of callus type watery and needs to subculture every 10 days and their color turns brown and loses vitality when delayed subculture for more than two weeks. So would excluded from subsequent experiments. and the adoption of callus induced from stem where the percentage of callus induction good at light conditions and which as will be discussed later , callus was friable kind and took almost two weeks for callus induction and color tend to off white, so it had been the adoption of this combination and the explant (stem) in subsequent experiments.

The culture of the explant separated from mature plants (leaves and stem) a negative effect, when cultured in MS media equipped with NAA and Kin, where did not callus induced from these explant at all concentrations and condition of light and darkness, so had been excluded in subsequent experiments. The MS media equipped 2,4- D + Kin had a positive effect on callus induction from leaves when cultured at light conditions , callus was friable type and color off white.

Percentage of callus induction from stem very small amount, the type was softy and color brown, therefore excluded from subsequent experiments. We rely the explant leave which cultured on MS media and equipped with 2,4-D + Kin, best explant for Callus induction. In spite of the fact that MS media equipped with 2,4-D at 2mg/l and Kin at1mg/l concentration was significantly superiority in the rate of callus induction, but behavior ability for regeneration will be discussed later when we discussed in the results.

The reason for the differences of the percentage of callus induction from different explant, of mature plants and seedling. To the difference in the type of explant and the totipotency from cells of different, explant. As well as the number of capable cell division, and internal content of the hormones prepare cells [17].Also callus induction is dependent directly on the concentration of growth regulators especially auxin and the size of explant used, it also depends on the environmental conditions such as temperature, light, humidity. In addition to the growth regulator that have a significant role in this process [18],Perhaps the reason for not returning callus induction of some explant, separated from the seedling or adult plants to the differentiation of cells and the lack of division, or maybe needs longer time for division and growth [19]. Evidenced by the current results of the study the response between the explant differences, which may be due to physiological factors related components content and hormonal differences [20)] or to the degree of maturation and differentiation of the vascular bundle of the cell, which depend on the transfer of nutrient absorbed from the culture media.

Percentage for callus induction from (stem seedling oleander and leaves of the mature plant)

The results of Table (1) show the NAA concentration significant effect in the percentage of callus induction from stem seedling ,which reaching the highest rate significantly of callus induction reaching to 40.8% at a concentration 1.5 mg /l , while the lowest rate of the percentage of callus induction reached to 6.6% at control treatment. Kin concentrations also influenced the percentage of callus induction, the higher rate arrived and significantly, to 56.2% at a concentration 1.5 mg/l, and less callus induction rate and significantly arrived to 0% in control treatment. And the impact of interference between the concentrations of Kin and NAA, significantly in the percentage of callus induction. The highest percentage of Callus induction reached and significantly, 88% at 1.5 mg/ l concentration for each of the NAA and Kin that consider the maintenance media, while reached the lowest rate of callus induction significantly 7% at a concentration of 1mg / l Kin and 0 mg / l NAA.

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Table 1: Effect of different concentration of NAA and Kin on percentage of callus induction from stem seedling of *Nerium oleander*

NAA / KIN	0	0.5	1	1.5	2	Average
0	0 o	0 o	0 o	0 o	0 o	0 d
0.5	0 o	15 lm	17 kl	25 ij	13 lm	14 c
1	7n	22Jk	39 fg	53 d	48 de	33.8 b
1.5	16 l	48 de	69 b	88 a	60 c	56.2 a
2	10 mn	40 fg	44ef	38 gh	33 hi	33 b
Average	6.6 d	25c	33.8 a	40.8 a	30.8 b	

As for callus induction from the mature oleander, plant's leaves .expects results table (2). that the highest rate of the percentage of Callus induction significantly reached to 65.4% at a concentration 2mg/l of 2,4-D,while the lowest rate for callus induction and significantly reached 7% at a concentration 0 mg/l of 2,4-D ,Kin concentration was significantly effect in the percentage of the callus induction. Which reached to the highest percentage rates and significantly, 54.4% at a concentration 1.5 mg/l of Kin. And reached the ratio to its lowest level significantly to 6% at 0mg/l concentration of Kin. The results show The same table that the inter action between the concentration of 2,4-D and Kin significantly effect in the percentage of callus induction, which reached their highest percentage significantly to 91% at a concentration 2 and 1 mg / l of 2,4-D and Kin respectively. And considered this combination was the maintenance media of callus. And reached the lowest rat significantly, 3% at a concentration of 0.5mg/l and 0mg / l 0f 2,4-D and Kin respectively. The presence of Auxin great importance in callus induction alone or with a Cytokinin, as working Auxin first on cellular wall disintegration and therefore cell division. Secondly metabolism of nucleic acids, particularly RNA, which leads to synthesis proteins which was necessary for the process of division and multiplication of cells. All of which leads to the growth and development of tissues in the plant and the formation of callus. In addition, the Cytokinin work on the metabolism of sugars and protein regulation Tubulin and thus stimulate cell division [21)] Good growth of callus in culture media get by the physiological balance between Auxin and Cytokinin. And increase the concentration of either of them over the other had a negative impact, on the growth of callus [22)] The addition of Auxin and Cytokinin to culture media necessary for callus induction, where Cytokinin worked in existence of Auxin as the key to start cell division. Perhaps the reason for the different response of explant cultured for callus induction, due to proportion of Cytokinin to Auxin added. may be due to the difference in the content of these inner parts of the interior of hormones and this in turn affects attainment of the ideal concentration for callus induction, from Auxin and Cytokinin or both when add to this culture media[23)]

Table 2: Effect of different concentration of 2,4-D and Kin on percentage of callus induction from leaf of mature *Nerium oleander*

2,4-D / Kin	0	0.5	1	1.5	2	Average
0	0 q	3pq	5p	9o	13mn	6e
0.5	0 q	10 no	33 J	59g	68 de	34 d
1	11 mno	26 l	45 i	78 c	91 a	50.2 b
1.5	14m	30 Jk	67 e	77c	84b	54.4 a
2	10 no	28 kl	55h	63 f	71 d	45.4 c
Average	7 e	19.4 d	41 c	57.2 b	65.4 a	

Fresh and dry weight of callus induced from stem seedling oleander and leaves of mature plant

The results of the two tables (3,4) that the concentration of NAA effect significantly in fresh and dry weight , for callus induced from stem seedling. the highest rate significantly reaching to 423.11 and 37.48 mg for fresh and dry weight respectively, at a concentration 1.5mg/l, while the lower rate value significantly 82.09 and 8.83 mg for fresh and dry weight, respectively at concentration 0 mg/l NAA. Also the concentration of Kin effects on fresh and dry weight, the highest rate significantly reaching to 590.77 and 71.04 mg fresh and dry weight respectively at 1.5mg/l concentration of Kin, arrived the less rate significantly arrived to 0mg of fresh and dry weight respectively in control treatment. And the impact of interference between the NAA and Kin concentration

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significantly in fresh and dry weight. while the highest rate significantly arrived to 820.27 and 96.67 mg for fresh and dry weight respectively, at a concentration 1.5mg / l of Kin and NAA, while the lowest rate significantly 103.65 and 11.53 mg for fresh and dry weight respectively at a concentration 0mg / l of NAA and 1mg / l of Kin.

Table 3: Effect of various concentration of NAA and Kin on fresh weight (mg) of callus induction from stem seedling of *Nerium oleander*

NAA / Kin	0	0.5	1	1.5	2	Average
0	0 n	0 n	0 n	0 n	0 n	0d
0.5	0 n	250.30 j	275.77i	351.63 g	228.13 k	221.16 c
1	103.65m	300.87 h	430.54 e	645.09 c	479.52 d	391.93 b
1.5	200.76 l	482.22 d	730.63 b	820.27a	719.99 b	590.77 a
2	106.06 m	363.88 g	395.70 f	298.58 h	203.71 l	273.58 c
Average	82.09 d	279.45 c	366.52 b	423.11 a	326.27 b	

Table 4: Effect of various concentration of NAA and Kin on dry weight (mg) of callus induced from stem seedling of *Nerium oleander*

NAA / Kin	0	0.5	1	1.5	2	Average
0	0 n	0 n	0 n	0 n	0 n	0 d
0.5	0 n	21.75 ijk	22.00 ij	25.48 ghi	18.61jkl	17.56c
1	11.53m	27.79gh	33.81ef	46.66d	37.30e	31.41b
1.5	16.90kl	69.85c	84.37b	96.67 a	87.44 b	71.04 a
2	15.75 lm	23.95hi	29.04fg	18.59jkl	15.81lm	20.62 c
Average	8.83 d	28.66 c	33.84b	37.48 a	31.83 b	

As for the results of fresh and dry weight for callus induced from leaves of mature plant. a higher rate had reached significantly to 240.2 and 51.49 mg for fresh and dry weight respectively at 2mg / l concentration of 2,4-D, table (5 ,6), while the less rate significantly reached to 24.2 and 5.65mg for fresh and dry weight respectively at 0 mg/l concentration of 2,4-D. Also the concentration of Kin significantly influenced, the highest rate significantly reaching of 190.6 and 93.63 mg for fresh and dry weight respectively at 1.5 mg / l concentration for fresh weight and 1mg/l concentration for dry weight, the less significantly value 22.2 and 3.57 mg for fresh and dry weight respectively at 0 mg /l concentration for Kin. The inter action between the concentrations of 2,4-D and Kin effect significantly of increasing the both weight. higher rate reached significantly 385 and 87.33 mg for fresh and dry weight Respectively, at 2mg/l concentration of 2,4-D and 1mg / l Kin, while the less rate significantly 8 and 0.41 mg for fresh and dry weight respectively at 0 and 0.5 mg / l concentration of 2,4-D and Kin respectively.

It was to increase the concentration of Kin within reach ideal concentration positive impact in increasing fresh weight for callus. May be because to the fact that cytokinin significant increase in cell division and especially meristematic cell, and this in turn leads to increase the size of various tissues of plant organ. Whether they would connected to plants mother or separated. and cultured in sterility culture media [24)] The reason for increasing the percentage of callus induction and increase the fresh weight , may be due for callus when increasing concentrations of 2,4-D because it affects the effectiveness of the enzymes and increases cell division and then increasing the formation of callus [25]. The addition of Auxin 2,4-D and NAA to the culture media, at concentration higher than the ideal concentration , may adversely affect the work of the enzymes which responsible for cellular wall building. Thus affect the mechanical properties and thus affect cell division and the formation of callus, which affect the outcome on the fresh and dry weight for callus [26)] The good growth callus in the culture media gets by physiology balance between Auxin and cytokinin . And increase either of them over the other lead to negative to affects the growth of the callus which reflected on the fresh and dry weight [22)]

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Table 5: Effect of various concentration of 2,4-D and Kin on fresh weight (mg) of callus induced from leaf of mature *Nerium oleander*

2,4-D / Kin	0	0.5	1	1.5	2	Average
0	0 o	8o	14o	34 mn	55 kl	22.2 c
0.5	0 o	36 mn	139 h	196 f	217 de	117.6 b
1	41 lm	90 j	178 g	239 c	385 a	186.6 a
1.5	58k	117i	219 de	226 cd	333 b	190.6 a
2	22 no	105 i	153 h	193 f	211 e	136.8 b
Average	24.2 d	71.2 c	140.6 b	177.6 b	240.2 a	

Table 6: Effect of various concentration of 2,4-D and Kin on dry weight (mg) of callus induced from leaf of mature *Nerium oleander*

2,4-D / Kin	0	0.5	1	1.5	2	Average
0	0 p	0.41 p	0.55 p	5.84 o	11.07mn	3.57 c
0.5	0 p	9.38 n	24.66 i	38.67 f	43.65 e	23.27 b
1	10.39 mn	14.93 l	29.81 h	55.71 c	87.33 a	39.63 a
1.5	15.31 k	19.63 j	32.77 gh	46.98 d	76.50 b	38.23 a
2	2.59 p	12.82 lm	18.92 j	33.26 g	38.91 f	21.30 b
Average	5.65 e	11.43 d	21.34 c	36.09 b	51.49a	

The effect of the concentration of BA and NAA in the emergence of shoot branches

Were cultured callus induced from the leaves separated from the mature plant oleander in the regeneration media equipped with a concentration of BA and NAA. The behavior of this callus that remained in the form of callus for the duration of the experiment, and did not distinction to the branches of the vegetation and all the interactions. So were excluded from subsequent experiments, perhaps the reason of this is due to we cultured the stems and roots grow discouraged in the media containing 2,4-D different concentration. the embryonic cells did not develop if cultured callus on the media containing 2,4-D [27)] As for the callus behavior induced from the stem seedling , results in table (7) showed that the inter action between hormones in maintenance media and concentration of BA in the regeneration media had an impact significantly on callus differentiation. The highest rate of number of shoot branches significantly arrived to 10.66 Branch at concentration 2mg/l of BA, while the lowest rate for the number of branches significantly reached 1.43 at 0.5mg/l. Callus did not differentiation at 0mg/l concentration of BA. The concentrations of NAA significantly effect in the rate of number of branches, the highest rate significant of number of branches 6.2 branch at a concentration 0.5 mg/l of NAA while the lowest rates significantly 3.2 at concentration 0mg/l of NAA, which did not different significantly by the number of branches at a concentration 1mg/l. The interaction between the concentration of BA and NAA impact significantly, increase the number of branches differentiated from callus. The highest rate of number of branches significantly reaching 14 branch at a concentration 0.5 and 2 mg/l for both NAA and BA respectively. While the lowest rates of number of branch significantly reaching 1.3 branch at concentration 1 and 0.5mg/l for both BA and NAA respectively. Concerning the effect of the concentration of BA had varied, depending on the concentrations but in general, many researchers had agreement the importance of BA in the regeneration and multiplication branches in plant culture [28]. Also the BA is known as one of the most cytokinin that have an impact in the division process, and growth compared to other cytokinin.. And this effectiveness is due to the internal structure of the molecule of cytokinin and the number of double bonds that were owned in a chain of side [29]. While allowing greater BA opportunity to link to other molecule on cultured media, the events of the desired effect as well as a BA had benzene ring made a prominent cytokinin used to push the plant towards branch formation [30] who found a positive relationship between BA concentration and number of shoot branches of each explant.

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Table 7: Effect of NAA and BA concentration on shoot numbers induced from stem callus of *Nerium oleander*

NAA / BA	0	0.5	1	Average
0	0 f	0 f	0 f	0e
0.5	0 f	3 e	1.3 ef	1.43 d
1	5 d	9 b c	6 d	6.66 b
2	8c	14 a	10 b	10.66 a
3	3e	5 d	2 e	3.33 c
Average	3.2 b	6.2 a	3.86 b	

Effect of IAA concentration in the percentage of rooting, number and length of roots

The shoot branches of oleander plant resulting from cultured callus in regeneration media not be root, which abled them transported into the soil, so it was cultured in rooting media containing different concentration of IAA. The results (Table 8) that the concentration 2mg/l of IAA had gave the highest value significantly percentage of rooting, the length and number of roots, which reached 88% 1.18 cm and 4.7 root/branch respectively. While the ratio arrived to their lowest rate significantly at a concentration 0.5mg / l, which reaching 32%, 0.3 cm and 1.5 root / branch for each of the percentage of rooting , the length and number of roots respectively, root didn't induced in the control treatment. From these results, it was clear that Auxin had important role in rooting process, as the content of Auxin in shoot culture an important role to induced formation (root primordial) from the base of plant branches cultured. The division of the cell of root origin of the roots cells, depends on the internal Auxin or added to the culture media. the physiological effects of Auxin ,representing an increase of cell division, and the conversion of mature cell specializing in base cell of shoots into meristematic cell and thus formed meristems roots, which division its cells to formed adventurous rooted [31].

Table 8: percentage of rooting, number of root, and root length from shoot cultured on MS medium containing different concentration of IAA

Concentration of IAA	The percentage of rooting	Number of roots	Root length (cm)
0	0 d	0 d	0
0.5	32 c	1.5 c	0.3 b
1	59 b	2.6 b	0.6 b
2	88 a	4.7 a	1.18 a
3	37 c	2.2 b	0.4 b

May be the reason of decrease the rate in the length and number of roots, increasing the concentration of IAA in the culture media to increase the idol Auxin concentration, which causing inhibition roots process to formed (root primordial) and thereby reducing the number and speed of root growth.

Effect of culture media in the percentage of success acclimatization of plantlet

Table (9) show results that the type of mix culture media, effect significantly in the percentage of success of acclimatization oleander plantlet. The media component of one size of sand and peat moos significantly superiority in percentage of success acclimatization, which reached the ratio to 79%, while the lowest rate of success acclimatization significantly reached to 10% in the media component only peat moos. Perhaps the reason for increasing the percentage of success acclimatization in the media component of sand and peat moos to the fact that the sand media well for the spread and growth of roots and that a proportion of the pat moos helped in retaining good percentage of moisture and provides some needed of mineral which was necessary to growth.

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Table 9: Effect of type culture on the percentage of successful plantlet acclimatization.

River sand : peat moos	The percentage of successful acclimatization
0:1	10c
1:0	19c
1:1	79a
1:2	45b

CONCLUSION

Explant stem separated from the seedling of *Nerium oleande*r ,which considered best explant for callus induction when cultured in MS medium equipped with Kin + NAA at 1.5 mg/l concentration for both. Moreover, this combination gave the highest rate of fresh and dry weight of callus, when cultured this callus induced from these combination in regeneration media, which supplied with NAA and BA at concentration 0.5 and 2 mg/l respectively gave the highest rate of the number of vegetative branches. these branches rooted when cultured in MS medium equipped with IAA at 2mg/l concentration and the rate of a successes acclimatization of these plantlets reached to 79% when grown in medium contain one size for each of the peat moos and river sand.

REFERENCES

[1] Kings bury, J. M. Poisonous plants of the United States and Canada Englewood Cliffs NJ prentice Hall .1964

[2] Hardin, J.W. and Arena, J. M. Human poisoning from native and cultivated plants, 2nd ed. King Sport, Tennessee, Duke, university press.1974

[3] Huxley, A.1992 The new Horticultural society dictionary of gardening. Vol.3, Mac Millan publication co. Inc. London. UK. Vol 3.

[4] Garima, Z. and Amla, B. 2010. A review on chemistry and pharmacological Activity of *Nerium oleander*. L. J. of chem. and pharmaceutical research. 2(6): 351 – 358.

[5] Vikas, G. and Payal , M. 2010. Phytochemical Potential of *Nerium oleander*: A Review. 1(3): 21 – 27.

[6] Valent, J. Oleanders, Physiotherapy - disease cure with plants Aldo Martello – Glunti , Firenze, Italy :1976 . PP.332 – 333.

[7] Karrunakaran, C. M. and Soundararajan, T. 2010. Micro propagation of *Nerium oleander* Through the mature pods. J. of Agriculture: science 2(2) : 181 – 193.

[8] Ibrahim, A., Khalifa ,S., Khafagi, I., Yossef, D., Khar, I and Mesbah, M. 2009. Enhasement of Oleandrin production composition and substrate feeding. Plant Bio

[9] Garima, Zibbu and Amla, Butra. 2012. In Vitro and In Vivo determination of Phenolic contents and antioxidant activity of desert plants of *Apocynaceae* family. Asian Journal of pharmaceutical and clinical research 5(1): PP.76 – 83.

[10] Soundarajan, T. S. and Karrunakaran, C. M. 2010. Micro propagation of *Nerium oleander* through the immature pods. J. of Agricultures science. 2(2): 181 – 193.

[11] Santos, I., Guimaraes, I., and Salema, R. 1994. Somatic embargo genesis and Plant regeneration of *Nerium oleander*. Plant Cell tissue. Org. cult.37: 83 – 86.

[12] Pal, D.; Gupta, S. K., and Singh, C. 1990. Organogenesis and plant regeneration In leaf callus of *Nerium oleander* L. Ad. Plant sci.3: 61 – 65.

[13] Hatzilazarou, S., C. T tooulos, A.S. Economou, N. Rifaki, P. Ralli . 2000. International Symposium on Acclimatization and establishment of micro Propagated plants, Acta Horticulture, 616.

[14] Inmaculada, Vila, Ester, Sales, Javier, Ollero, Jesus, Munoz – Bortomeu, Junan segura, and Isabel, Arrillaga. 2010. Micro propagation of oleander *Nerium oleander* L. Horticulture Science. 45(1): 98 – 102.

[15] Murashige, T. and Skoog, F.1962. A revised medium for rapid growth and bio Assays with tobacco tissue culture. Physio. Plant.15: 473 – 497.

[16] Glaser, A. and Biggs, C. Introduction to statistical methods in Gen Stat. VSN. International Hemel. Hemel Hempstead, 2010.UK.

[17] Acquaah, G. 2004. Under Standing biotechnology Pearson education. Inc. U.S.A.

[18] Fernandez, M. T., Fuey, M. A., Ciraldez, R. and podriguez, R. 1990 In Vitro Morphogenetic Responses on seedling, Mature and immature (*Phaseolus vulgaris*). Embryo. Abs. VII the International confers on plant Tissue and cell Culture IAPTC. Amsterdam.

[19] Salih, S. M. and Al-Mallah, M. K. 2006. Plant regeneration from in vitro leaf and Stem tissue culture of *Solanum nigrum*. Dirasat Agriculture .sci. 27(1): 64 – 71.

[20] Trigiano, R. N. and Gray, D. J. (2000). Plant tissue Culture concepts and Laboratory exercise, CRC press LLC. Printed in the U.S.A. PP.283.

[21] Mahesh, S. 2008. Plant molecular biotechnology. New Age international (P) Ltd. 1st Edit, New Delhi, India. PP.49 – 51.

[22] Mineo, L. 1990. Plant tissue culture techniques', C. A. Gold Man Editor, PP.151 – 174.

[23] Goodwin, M. 1985. Introduction to plant Biochemistry. (2nd. Ed). Pergamon press.New York. P: 149.

[24] Delloloio, R. 2007. Cytokininus determine *Arabidopsis thaliana* root meristem Size by controlling cell differentiation. Curr. Biol., 17: 678 – 682.

[25] Bezo, M. and Stefunova, V. 2001. Indirect regeneration of *Hypericum* SP. under In vitro conditions, Acta phytoteachnicate zootechnica, 4 : 277 – 279.

[26] Taiz, L. and Zeiger, E. 2002. Plant physiology, 3rd Ed Sinauer. Sinauer Associates Inc. U. S. A., PP.283 – 308.

[27] Martins, I. S. and Saundahl, M. R. 1985. Early Stage of somatic embryo Differentiation from callus cell of bean of *Phaseolus vulgaris* growing in ligaid Medium. Hort. Abs. 1985. (5) 4: (2621).

[28] Wang, K., Edward, C.; Yeung, T. and Hisn, Y. 1992, Multiple shoot formation From mature cotyledon and cotyledonary nod of the bean *Phaseolus vulgaris*. In Vitro culture technique. Chinese Agro. J. 2: 155 – 167.

[29] Krishnamoorthy, H. N. Plant growth substances including application in Agriculture. Tata McGraw – Hill, New Delhi, 1981. PP.214.

[30] Fonnesbech, m. 1972. Growth hormones and propagation of Cymbidium in Vitro. Physio. plant.27: 310 – 316.

[31] Audus, L. J. Plant growth substances. 2nd. London, Leon and Hill Ltd. P.553.1959