Advances in Environmental Biology, 8(7) May 2014, Pages: 2223-2230

AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/aeb.html

The Effect of Various Concentrations of Iba and Naa on the Rooting of Semi Hardwood Cuttings of A*zalea Alexander* l.

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ARTICLE INFO

Article history:
Received 26 March 2014
Received in revised form 20 April 2014
Accepted 26 May 2014
Available online 10 June 2014

Key words:
Indole butyric acid (IBA), Naphthalene acetic acid (NAA), rooting, Semi- hardwood cuttings, Azalea alexander L.

ABSTRACT

In order to study the effect of various concentrations of Indole butyric acid (IBA), Naphthalene acetic acid (NAA) and mixture of these two hormones (IBA+NAA) on the rooting of semi- hardwood cuttings of *Azalea alexander* L, an experiment was carried out in a greenhouse around Salmanshahr town in 2014. The required semi- hardwood cuttings were prepared from the mother plants (stalks) planted in the bed of greenhouse and lacked the shoot. This experiment was executed in the mould of a complete randomized design with 3 replications and 16 treatments. In this research, the treatments were prepared to include hormone of Indole butyric acid in four levels of: (0, 1000, 2000 and 3000 mg/L), Naphthalene acetic acid in four levels of: (0, 1000, 2000 and 3000 mg/L) and mixture of these two hormones (IBA+NAA) with concentration of: (1000 +1000 , 2000+1000, 3000+1000, 1000+2000, 2000+2000, 3000+2000, 1000+3000, 2000+3000and 3000+3000 mg/L), and the bottom part of the cutting was dipped into above solutions for 5 seconds. The results showed that the maximum percentage of the rooted cuttings, number of root, fresh weight and dry weight belonged to IBA treatment with concentration of 3000 mg/L, NAA treatment with concentration of 2000 mg/L and (IBA +NAA) / mixed treatment with concentration of 1000+2000 mg/L. The highest length of root belonged to IBA treatment with concentration of 3000 mg/L. The maximum number and length of root belonged to IBA treatment with concentration of 3000 mg/L, NAA treatment with concentration of 1000 mg/L and (IBA+NAA) mixed treatment with concentration of 2000+2000 mg/L, and the best speed of rooting belonged to IBA treatment with concentration of 3000+3000 mg/L. Finally, the best treatment belonged to (IBA+NAA) / mixed treatment with concentration of 1000+2000 mg/L.

To Cite This Article: Maryam Mohana, Ahmad Majd, Sayeh Jafari, Shadi Kiabi, Maryam Paivandi., The Effect of Various Concentrations of Iba and Naa on the Rooting of Semi Hardwood Cuttings of Azalea Alexander l.. ***Adv. Environ. Biol., 8(7),*** *2223-2230, 2014*

INTRODUCTION

Azalea is a plant which belongs to family of Ericaceae and genus of Rhododendron. It is a shrub and has alternate leaves which are often collected in the ending part of branch [6]. The leaves are deciduous or perennial. It's flowers in the different colors are red, violet, white, pink and yellow. It's height reaches 1.5 meter too. This plant favors complete sun or penumbra (light and shade). *Azalea* prefers the low PH leading to increase of the plant's access to iron and magnesium. In feeding of *Azalea* for powerful vegetative growth, supply of enough quantities of nitrogen in of importance. Supply of these amounts must not be continued until formation of flower because continuation of vegetative growth leads to lack of formation of flower. Soil's PH is an effective factor on the feeding of nitrogen and low PH prohibits from activity of the organisms producing nitrogen and prevents from conversion of ammonium into nitrate, but it decreases activity of the decomposer microorganisms and leads to stabilization and unusability of nitrogen for plant. For this reason, the *Azaleas* must be planted in a bed with the high levels of organic substances and low PH [19].

Appropriate soil for this plant is peat and soil's drainage must be excellent. Proper PH to plant Rhododendrons encompasses a range from PH5 to PH6 [21].

Azalea is from the native plants of china and Japan. About a century ago, it would plant in the Greenhouse in the U.S.A, but it has been turned into a plant with high price due to slowness of growth and much cost of production [6].

Considering available facilities or economical situations, ornamental plants are reproduced by various method. If desired plant of producer is obtained from seed within a short time interval, then, increase/

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proliferation by seed can be counted as a cost – effective method. In most cases, production of ideal plant from seed in not possible because most ornamental plants are heterozygote and their seeds produce various plants.

In addition to this, some plants, due to genetic reasons (incompatibility between pollen grain and gynoecium, polyploid) or undesirable environmental conditions (such as inappropriateness of temperature and relative humidity for pollination) don't produce seed and the vegetative reproduction must be used to proliferate them.

To plant cuttings is one of the most common methods of vegetative reproduction which is, today, used extensively due to low cost. But, problem is here that all plants can not be reproduced by cuttings. Based Upon this, these plants are identified by us as hard rooting plants. Today, reproduction by foliate semi hardwood cuttings has been taken into consideration as a turning point in the affair of development of the new grassy or woody cultivars and species [9].

Culture medium is a factor which influences on the percentage of cuttings which are to be rooted and kind of the root created on it. Cultivation environment must have enough humidity and oxygen and is free from the pathogenic factors.

Reproduction of *Azalea alexander* L. performs hardly because it is attacked by the pests and insects.

In Iran, *Azalea alexander* L. is kept in the warm and wet greenhouses. Since this ornamental plant don't produce seed in the greenhouse conditions. It's reproduction, based on this principle, usually performs asexually and through cutting of semi- hard wood stalk. This method is a favorable method for production of the new plant and it's advantage is to produce the plants like the mother stalk. In it's increase or proliferation through cutting of stalk, a high percentage of cuttings is decayed and destroyed. Therefore, in order to improve reproduction of cutting, usage of auxin hormone seems to be necessary because this hormone assists rooting of *Azalea's* semi- hard wood cuttings noticeably. Treatment of the cuttings with various substances to stimulate the in adventitious root has as ancient history. For example, placement of budding seed of grains in the cleft of the cuttings' bottom part which is an ancient method and people of the Middle East and ancient Europe used it. This method has the scientific foundation because budding seeds produce Indole acetic acid which is natural auxin.

The auxins used in the rooting are usually synthesized auxins of IBA and NAA.These two auxins has maximum effect on the stimulation of production in adventitious roots [8].

Possibly, IBA is the best substance for general usage because it is non- poisonous in an extensive concentration and effective on many herbal species [23]. Search for finding of new auxins which have stimulant effect on the rooting remains to continue yet. From viewpoint of economical profit and much inclination of the floriculturists toward it, *Azalea alexander* L. is of great importance inside and outside of the country and a lot of studies have been carried out on it, but there is not available report regarding effect of rooting hormones of IBA and NAA on the ontogeny aspects of this plant. For this reason, performance of a research specifying role and effect of chemical substances on the rooting of *Azalea alexander's* cuttings in the greenhouse conditions seems to be necessary and can have scientific and applied results.

In the various mutants of root, role of auxin hormone in the beginning of growth and development of root from initial stages of embryogenesis has been clarified. Out of the genes, for example, have been identified in this connection, is gene family of PIN FORMED which includes 8 genes from PIN_1 to PIN_8 [1].

When *Azaleas* are grow under favorable conditions diseases are less of a problem. When they do occur they can often be traced back to their environment [25].

Reducing plant stress, such as transplant shock or drought, is the most important control measure. Making sure that plants are shaded from the hot afternoon sun is also important. Heat and drought stress in particular have been shown to favor this disease [7].

Azaleas benefit from a sheltered location that provides protection from the direct afternoon sun and the winter winds [11].

MATERIALS AND METHODS

In order to study effect of various concentrations of Indole butyric acid (IBA), Naphthalene acetic acid (NAA) and mixture of these two hormones (IBA+NAA) on the rooting of semi- hardwood cutting of the *Azalea alexander* L., an experiment was carried out in a greenhouse around the Salmanshar. The required semi hard wood cuttings were prepared from mother plant (stalk), which were 15-20cm in length and planted in the bed of greenhouse and lacked the shoot.

In order to carry out this research, the complete randomized design with 16 treatments in 3 replications and, in each replication, 10 cuttings were used.

The treatments applied in this experiment included 4 levels of IBA with concentration of 0, 1000, 2000 and 3000 mg/L, 4 levels of NAA with concentration of: (1000 +1000 , 2000+1000, 3000+1000, 1000+2000, 2000+2000, 3000+2000, 1000+3000, 2000+3000and 3000+3000 mg/L).

For treatment of the cuttings, almost 2.5 cm of their bottom was treated collectively in the beaker containing each one of the treatments for 5 seconds and, then, each cutting was planted separately in the middle part of the vases containing the peat.

After the root organogenesis, attributes, including percentage of rooted cuttings, speed of rooting, fresh weight, dry weight, number and length of root were evaluated. By separate extraction of each replication and counting of number of the cuttings in which rooting was carried out, percent of rooted cuttings was specified.

Roots of each replication were cut from place of nodule separately and weighed. The obtained number was regarded as fresh weight of root for each replication. In order to obtain the root's dry weights, samples were dried in the 105^0 C oven for 24 hours and, then, weighed. Number of roots were counted and taken note by extraction of all roots from cultivation environment. Length of all roots was measured by ruler. Analysis of data variance and test of comparison of average through Turki test was conducted using SAS software and drawing the diagrams by Excels soft ware.

Results:

Analysis of the data variance (table1) shows that effect of treatments on the average of squares of attributes in the 1% level of tuki test has had significant difference, and the evaluated attributes have been affected by exercised treatments.

Table 1: Significance of effect of the treatments in level of 1%.

Average of Squares (MS)						Freedom degree	Sources of changes
Speed of rooting	Dry weight	Fresh weight	Length of root	Number of root	Percentage of rooting		
47/5731**	0/075817**	0/3899**	121/674**	1502/9875**	984/313**	15	treatment
9/527	0/02087533	0/000053	0/07531	3/25	0/5325	32	Error
17/45	14/73	12/5	9/89	6/02	11/76	CV(%)	

Number of root:

1. Number of roots in each cutting is the highest quantity in the treatment of mixture of two hormones (IBA+NAA) with concentration of (1000+2000) mg/L and its difference with control treatment is significant in the 1% level there is not a difference in the level of 1% between the mixed treatments (IBA+NAA) with concentration of (1000+2000) mg/L and mixed treatment of (IBA+NAA) with concentration of (2000+3000) mg/L. Also, a significant difference in the 1% level was not observed between the mixed treatment (IBA+NAA) with concentration of (1000+ 3000) mg/L and / with the mixed treatment of (IBA+NAA) with concentration of (2000+3000) mg/L.

Comparison of the root number of IBA having the concentration of 2000 mg/L with root number of IBA having the concentration of 3000 mg/L suggests the significant difference between these two treatments so that increase of IBA's concentration from 2000 mg/L to 3000 mg/L was led to increase of root number.

Comparison of root number of 3 treatments of (IBA 1000), (IBA 2000) and (IBA 3000) with control suggests significant difference among these 3 treatments with control treatment.

Number of root in each cutting is the highest amount in the treatment of NAA with concentration of 2000 mg/L, and is showed a significant difference in 1% level with NAA through concentration of 1000 mg/L and NAA through Concentration of 3000 mg/L and with Control. (Figure 1)

"Fresh weight of root":

Study of analysis of the data Variance related to the fresh weight of root showed that fresh weight of root in two treatments of NAA with concentration of 1000 mg/L and NAA with concentration of 2000 mg/L is higher than that of control which is significant statistically. In this group, NAA with concentration of 3000 mg/L has not a Significant Difference with control. Comparison of root's Fresh weight in the treatment with IBA in each three concentrations of 1000, 2000 and 3000 mg/L has a significant difference with control in level of 1%. By increase of IBA'S Concentration from 1000 mg/L to 2000 mg/L, an increase and significant difference was observed in the Fresh Weight. Also, by increase of the concentration from 2000 mg/L to 3000 mg/L , an increase and significant difference was observed in the fresh weight.

Among treatment of mixture of two hormones, the highest Fresh weight related to the mixed treatment (IBA+NAA) with concentration of(1000+2000) mg/L, which has a significance difference with control in 1% level(figure 2)

"Dry weight of root":

Comparison of average of attribute of root's dry weight showed that maximum rate of the root's dry weight has been related to treatment of mixture of two hormones with concentration of (1000+2000) mg/L.

Root's dry weight in each cutting in the treatment of IBA with concentration of 3000 mg/L is the highest quantity and its difference with other treatments of IBA and control treatment is significant in 1% level.

Root's dry weight in the treatment of NAA with concentration of 2000 mg/L is the highest quantity which showed a significant difference with control treatment in the 1% level. (Figure 3)

"Length of root in each cutting":

Study of analysis of the data variance related to attribute of root's length shows that, among the mixed and non-mixed hormonic treatments, the highest length of root relates to IBA with concentration of 2000 mg/L which has a significant difference with other IBA and control treatments in level of 1%.

Regarding treatment of NAA, the longest length of root relates to NAA with concentration of 1000 mg/L which there has been a significant difference in level of 1% between this treatment with treatment of NAA with concentration of 2000 mg/L and treatment of NAA with concentration of 3000 mg/L. A significant difference was not observed between Treatment of NAA with concentration of 3000 mg/L with Treatment of control.

Regarding treatments of mixture of two hormones, the longest length of root has been related to treatment of (IBA+NAA) with concentration of (2000+2000) mg/L.(Figure 4)

"Percentage of rooting":

The IBA hormonic treatments in each three levels of 1000, 2000 and 3000 mg/L have been led to increase of rooting which has had a significant difference with control treatment in level of 1%.

In each three levels, NAA hormonic treatments have been led to increase of percentage of rooting in the cuttings which have a significant difference with control treatment in the 1% level. In the mixed treatments, the best treatment was treatment of mixture of (IBA+NAA) with concentration of (1000+2000) mg/L which had a significant difference with other treatments and control treatment in level of 1%.(Figure 5)

"Speed of rooting":

Study of analysis of the data variance related to this attribute shows that the maximum speed of rooting has been related to treatment of mixture of(IBA+NAA) with concentration of(3000+3000) mg/L. Speed of rooting in each cutting has been the highest amount in the treatment of IBA with concentration of 3000 mg/L and treatment of NAA with concentration of 3000 mg/L which showed a significant difference in 1% level.(Figure 6)

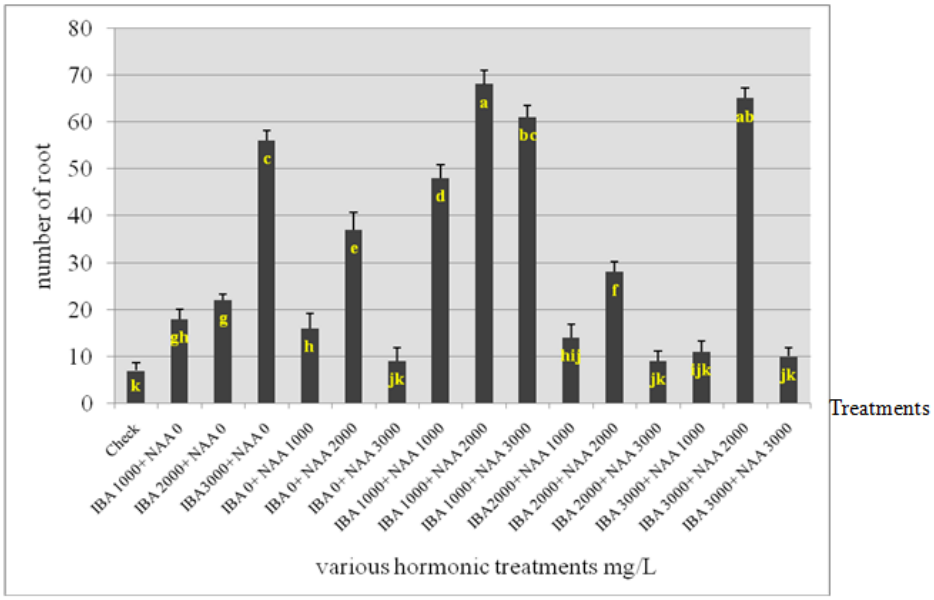

Fig. 1: Average of effect of the treatment on the number of root The columns which have joint letters have not a significant difference in level of 1%

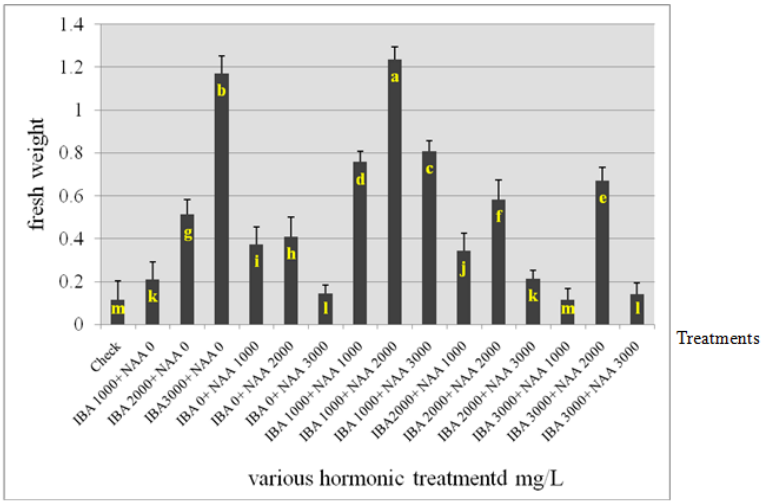

Fig. 2: Average of effect of the treatment on the fresh weight of root The columns which have joint letters have not a significant difference in level of 1%.

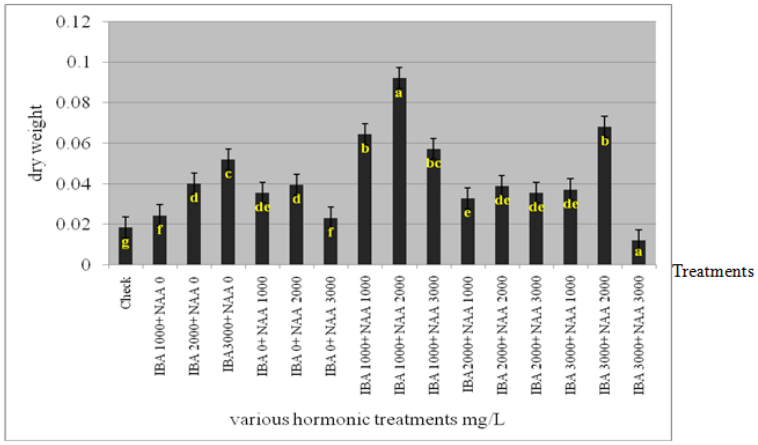

Fig. 3: Average of effect of the treatment on the dry weight of root The columns which have joint letters have not a significant difference in level of 1%.

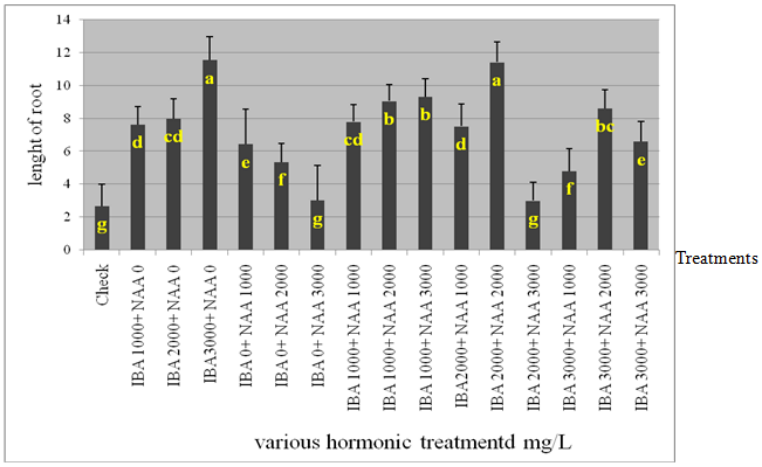

Fig. 4: Average of effect of the treatment on the length of root The columns which have joint letters have not a significant difference in level of 1%.

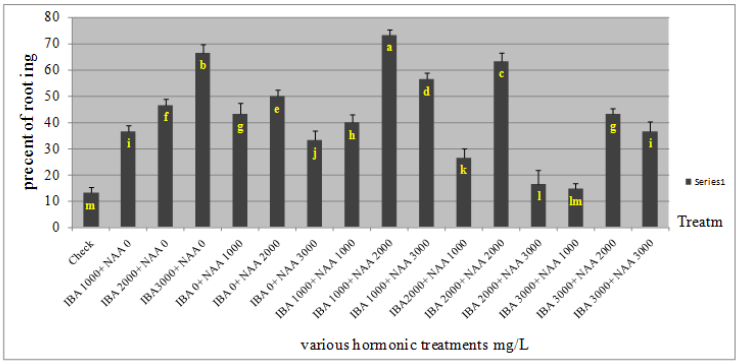

Fig. 5: Average of effect of the treatment on percentage of rooting The columns which have joint letters have not a significant difference in level of 1%.

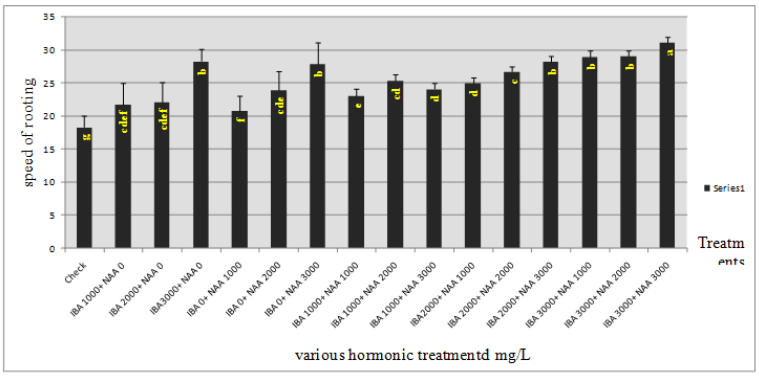

Fig. 6: Average of effect of the speed of rooting The columns which have joint letters have not a significant difference in level of 1%.

Discussion:

Regarding effect of treatment of the Naphthalene acetic acid(NAA) on the rooting of *Azalea Alexander* L. , results show that difference of treatments of Naphthalene acetic acid(NAA treatment) with control treatment is significant, and increase of concentration of this hormone not only has not been led to increase of rooting percentage, but also has been led to it's decrease as well. Difference of rooting percentage among three levels of NAA became significant. This subject corresponds with results of some researchers. [4].

In 2007, kigomo Bernard reached this conclusion that NAA is the best regulator substance in order to root *Bamboo* cutting and has suggested concentration of 100 mg/L.[14].

In connection with effect of the treatments on the rooting of the *Azalea alexander's* cuttings, results reveal that application of Indole butyric acid(IBA) in three levels of 1000,2000 and 3000 mg/L has been led to significant increase in rooting percentage compared to control treatment which corresponds with results of researches of other researchers.[5,13].

On the basis of results obtained from this experiment, treatment with hormones of indole butyric acid(IBA) and Naphthalene acetic acid(NAA) has influenced significantly on the attributes, including rooting percentage, number of root in the cutting, length of root, fresh weight and dry weight of root and speed of rooting in each cutting. Reason for positive effect of this substance on the rooting can be attributed to effect of the auxin on the stimulation of initial/first cellular division of the root's initiator [13].

In 2004, Belight *et al.*, studied various concentrations of IBA and NAA on the rooting of cuttings of the *Camellia japonica* and observed that concentrations of 3000 mg/L of IBA and(2000 mg/L of IBA+1250 mg/L of NAA) have been led to increase of percentage of rooting of the cuttings at a noticeable rate. Also, they expressed that employment of synthesized auxin with high concentration on the cuttings of stem can prevent from growth of the shoots and even growth and development of the branch and Foliage [12]. These results correspond with results obtained by Mirsolaimani and Rahemi in 2008 based on that increase of concentration of IBA and NAA up to concentrations of 1500 and 2000 mg/L, respectively, has been led to increase of three attributes of number, length and fresh weight of branch/foliage (shoot) and to its decrease again in higher concentrations.

These researchers believe that the high concentrations of auxin can be led to destruction of tissues of the cutting's bottom [16].

Other than auxin, other factors play a role in the rooting of cuttings. Synchronized with stimulation of rooting by auxin, transfer of carbohydrates from leaf to root assists rooting noticeably so that sugars, compounds containing nitrogen, phenolic mixtures and other cofactors are effective on the rooting of cutting [13].

Hormonic treatment of auxin leaves three various effect on the rooting of cuttings:

1- Acceleration in the appearance of root leading to increase of average of root's length.

2- Increase of number of adventitious roots.

3- Increase of percentage of rooted cuttings [8]. In 2010, Karamy and Salehi, through studying the effect of two kinds of synthetic auxin, NAA and IBA on the rooting of cuttings of *Tecomella undulata* in two seasons of the late of autumn and winter, expressed that a significant difference was observed between NAA and IBA hormones in the rooting. NAA with concentration of 3000 mg/L and IBA with concentration of 4000 mg/L had the highest percentage of the rooting.[12].

In 2013, Noori, by study of effect of NAA and IBA on the root generation of cuttings of *Ficus benjamina's* stalk, declared that the best concentration for rooting of *Ficus benjamina* is NAA with concentration of 1000 mg/L and IBA with concentration of 2000 mg/L which it has been shown that the various hormonic concentrations can influence on the cutting's rooting noticeably [18]. Regarding the treatments of mixture of two hormones, the best rooting was related to (IBA+NAA) with concentration of (1000+ 2000) mg/L which this result corresponds with the results obtained from the experiment carried out by Bagherian in 2010 who had used the various concentrations of two types of synthesized auxin on some cuttings in *Deracaena fragrans* L. [4].

In connection with superiority of treatment of Indole butyric acid (IBA) with concentration of 2000 and 3000 mg/L compared to some mixed treatments observed in some cuttings of the *Azalea alexander* L. , the results are similar to a research conducted by tewchounjeu *et al.*, on the pharmaceutical species of *pausinystalia johimbe* in 2004 [24].

Regarding the differences observed in quantity and quality of rooting and, also, harmonic treatments and environmental conditions, perhaps the less-identified internal factors, including genetic factors can be considered to contribute [15].

In a research accomplished by Shahmohammadi et.al on the rooting of *Dracaena sanderiana* L. in 2009, results showed that the highest number of the root was observed in the treatment of leaf-bearing cutting with concentration of 100mg/L of the NAA .

In vitro culture of orchid (*Denrobuim-nobil*), rooting was carried out by use of two NAA and IBA hormones. Increase of percentage of rooting, number of root and length of root in IBA treatment with concentration of 2 mg/L were more effective than NAA treatment. Also, in the concentrations higher than about 3 mg/L of IBA and NAA, weaker results of rooting were obtained [3].

In an experiment performed by Kroni, *et al.* in 2005 on the semi-hardwood cuttings of *Rose*, *Koelreuteria paniculata*, *pittosporum tobira* and *Corylus avellana*, the obtained results regarding the tested plants suggested increase of number of root in the cutting and increase of percentage of rooting compared to control treatment. The only exception was *Pittosporum tobira* which had 100% of rooting in most treatments, which this can be due to existence of sufficient internal rooting factors in the cuttings of this plant [4]. Results of this study is in conformity with the results obtained from this test.

In 2005, Moallemi and Chehrazi showed that treatment of cuttings of *Bougainvillea glabra* with the hormone caused that length of root in each cutting compared to treatment of control is to be increased significantly but, a significant difference was not observed among the various concentrations of IBA and NAA from this view point [17]. Which is different slightly with the results obtained from this experiment.

Conclusion:

Totally, it can be said that concentrations of 3000 MG/L of indole butyric acid, 2000 mg/L of Naphthalene acetic acid and mixture of two regulators of 1000 mg/L of Indole butyric acid and 2000 mg/L of Naphthalene acetic acid were the most appropriate treatments in order to root the semi-hard wood cuttings of the *Azalea alexander* L. under available conditions.

ACKNOWLEDGEMENT

All persons who assisted us to carry out this practical project are acknowledged manimally.

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