An Evaluation Study on the Effects of Sodium Alginate and Irradiated Sodium Alginate on the Growth of *Acokanthera oblongifolia* Hochst

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ABSTRACT

Two experiments were carried out at Antoniades Research Branch, Ornamental Plants Research and Landscape Gardening Department Horticulture Research Institute, A.R.C. Alexandria, Egypt during the two successive seasons 2014/2015 and 2015/2016 to investigate the effect of sodium alginate (SA) and irradiated sodium alginate (ISA) on seed germination and the growth of *Acokanthera* plants. The first experiment was to investigate the effects of (SA and ISA) on seed germination; the seeds of *Acokanthera* were soaked in seven solutions 50 ppm SA and different concentrations of ISA [0.0 (distilled water), 50, 75, 100, 125 and 150 ppm] for 24 hours, The treatment 50 and 125 ppm ISA significantly improved germination rate and percentage. The second experiment was to study the effect of the foliar spray of SA and ISA on *Acokanthera* plants with the same concentrations used in the first experiment the results showed that all treatment of ISA caused an increment in most studied characters and the treatment 50 ppm ISA caused the highest significant increase in all vegetative growth parameters (plant height, numbers of leaves, fresh weight, dry weight, leaf area and stem diameter), root characters (root volume and root dry weight), chlorophyll and carbohydrate content.

Key words: *Acokanthera oblongifolia*, Sodium Alginate (SA), irradiated sodium alginate (ISA).

INTRODUCTION

*Acokanthera oblongifolia* Hochst. (Synonyms *Acokanthera spectabilis*, *Carissa oblongifolia*, *Toxicophloea spectabilis*) common names (Bushman’s poison, poison bush, poison tree and a winter sweet) belongs to family Apocynaceae. It is a medium to a large woody shrub with an attractive hard dark green leaves. Clusters of pinkish white, sweetly scented flowers are borne in late winter and spring and they are followed by a large plum colored berry-like fruits which relished by birds. The Bushman's poison is a hardly drought and frost resistant evergreen shrub that tolerates full sun or shade and also does well as a container plant. (Arnold & De wet 1993 and Pooley 1993).

Sodium alginate is a natural polysaccharide. It is the sodium salt of alginic acid. Its formula is NaC₆H₇O₆, which is derived from the cell walls of brown algae. It can be degraded from macromolecules to micromolecules which is known as oligomers by using gamma irradiation (Luan et al., 2003). This oligomers are used in the biomedical and agriculture field (El-Mohdy, 2017). Irradiated sodium alginate has many physiological and biological activities on plants (Mollah et al., 2009). These activities include promotion of plant growth, seed germination, shoot elongation and root growth (Yonemoto et al., 1993; Natsume et al., 1994; Hu et al., 2004).

The aim of this study was to investigate the effects of sodium alginate (SA) and irradiated sodium alginate (ISA) on improving seed germination and growth of *Acokanthera oblongifolia* plants.

MATERIAL AND METHODS

The present study was carried out during two successive seasons of 2014/2015 and 2015/2016 at Antoniades Research Branch, Ornamental Plants Research and Landscape Gardening Department. Horticultural Research Institute, A.R.C. Alexandria, Egypt.

Two experiments were done in this study; the first one was to investigate the effect of sodium alginate (SA) and irradiated sodium alginate (ISA) on seed germination of *Acokanthera* plants and the second one to investigate the effect of foliar spray of SA and ISA on the growth of *Acokanthera* seedlings.

Gamma rays which are used for this experiment were generated from Cobalt-60 source at the National Center for Radiation Research and Technology, Atomic Energy Authority Nasr City, Cairo, Egypt. SA sample was irradiated with 520 Kilo Gray at the rate dose 2Kilo Gray (K Gy) gamma rays. It was sealed in a glass vial with atmospheric air. 50 ppm un-irradiated sodium alginate (SA) and different concentrations of irradiated sodium alginate (ISA). [0.0 (distilled water), 50, 75, 100, 125 and 150 ppm] were finally prepared using distilled water.

The first experiment: The effect of sodium alginate (SA) and irradiated sodium alginate (ISA) on seed germination of *Acokanthera* plants:

On the 20th of March 2015 thirty two seeds of *Acokanthera* plant were soaked for 24 hours in seven solutions; deionized water (control), (50 ppm (SA) and [50, 75, 100, 125 and 150 ppm ISA]. The
seeds were planted in a germination tray containing sand soil one tray for each treatment.

The following data were recorded:

1- Germination rate (GR)

It was calculated according to the following formula (Mahmoud, 2013):

\[
\text{Germination rate (GR)} = \frac{a + (a + b + c) + \ldots + (a + b + c + m)}{n(a + b + c + m)}
\]

Where a, b, c are the number of germinated seeds in the first, second and third count, m is the number of germinated seeds in the final count, n is the number of counts.

2- Germination percentage (%):

It was calculated according to the following formula (Mahmoud, 2013):

\[
\text{Germination percentage} = \left( \frac{\text{number of germinated seeds}}{\text{total seed number}} \right) \times 100
\]

This percentage was calculated after 60 days of planting.

The second experiment: The effect of sodium alginate (SA) and irradiated sodium alginate (ISA) on the growth of Acokanthera plants:

On 18th and 23rd of June on the two seasons 2014/2015 and 2015/2016, respectively. Nine weeks seedlings of Acokanthera plants (2 pairs of leaves) were planted in 14 cm plastic pots containing a mixture of sandy and clay soil at the ratio of (1:1 by volume).

After six days of planting the seedling were sprayed with hand sprayer with the following treatments: Deionized water (control), 50 ppm SA, 50, 75, 100, 125 and 150 ppm ISA. The treatment repeated weekly for five months. On November 2014 and 2015 in the first and second season; respectively the treatment of ISA stopped for four months. On March 2015 and 2016 in the first and second season the foliar spray of ISA continued weekly for four months. The experiment terminated on the first of June on 2015 and 2016 for the first and second season respectively.

The following data were recorded:

1-Relative plant height growth rate (cm month⁻¹):

It was calculated according to the formula of John et al., 2006

\[
\text{Relative height growth rate} = \frac{\ln H_2 - \ln H_1}{T_2 - T_1}
\]

Where H2 is the plant height at the end of the experiment and H1 is the plant height at the beginning of experiment. T1 is the first time and T2 second time.

2- Vegetative growth characters: Plant height (cm), number of leaves/plant, vegetative growth fresh weight (g), vegetative growth dry weight (g), leaves area (cm²) and stem diameter (mm).

3- Root characters root volume (cm³) and root dry weight (g)

4- Chemical analysis: Chlorophyll a and b content (mg/g fresh weight) was determined according to Moran, 1982, total carbohydrate content (%) in the leaves of the plant according to Hedge and Hofreiter 1962 and anthocyanin was determined by placing leaf discs (0.9 cm² total) in 3 ml acidified methanol with 10 ml concentrated HCL/L for 2 days at 4 °C. Light absorbance of the methanol extracts were determined at 530 and 657nm Anthocyanin concentration was calculated using the following formula of Mancinelli et al.,(1988): Ant = A 530 – 0.25 (A 657).

Statistical Analysis

The experiment layout was designed to provide randomized complete blocks design (RCBD) which is containing three replicates, each replicate contained seven treatments. Three plants were used as a plot for each treatment in each replicate. The means of the individual factors were compared by L.S.D. at 5% level of probability according to Snedecor and Cochran, 1989.

RESULTS

The first experiment: Effect of SA and ISA on seed germination of Acokanthera plants:

Figure (1) illustrated that highest germination rate was obtained after soaking the seeds in 50 and 125 ppm ISA, they are both more than 0.5. For germination rate after 60 days of planting. Figure (2) showed that all treatments caused an increment in germination percentage compared to control plant which was 59.38% and the highest germination percentage was obtained by seed soaking in 50, 125 and 150 ppm ISA which were more than 90%.

The second experiment: Effect of SA and ISA on the growth of Acokanthera plants:

1-Relative height growth rate

Figure (3 and 4) illustrated that the highest relative height growth rate was obtained by application 50 ppm ISA which was 0.102 cm month⁻¹ in the first season and more than 0.092 in the second one.

2- Vegetative growth parameters

Data in Table (1) showed that there was a significant increase in all studied vegetative growth parameters after application of all ISA treatments and the spray of 50 ppm un-irradiated sodium alginate resulted in promotive effects on all vegetative growth parameters. The highest plants were obtained after application of ISA at 75 ppm by 50.90% and 78.94% as compared to control in both seasons respectively. An exceed of number of leaves by 38.3% and 97.25% in the first and second season respectively compared to control plants was obtained by foliar spray of 50 ppm ISA, this
treatment caused the heaviest fresh weight by 82.31% and 98.88 % and heaviest dry weight by 102.3% and 186.13% compared to control in both season respectively. For leaves area the treatment 75 ppm ISA caused the highest increase by 82.86 % in the first season and the treatment by 50 ppm ISA 108.8 % in the second one compared to control. Also, the highest increase in stem diameter was obtained by the treatment 75 ppm ISA by 22.56 % and the treatment 50 ppm ISA by 43.12 % in the second season compared to control.

Figure 1: Effect of different treatments of sodium alginate and irradiated sodium alginate on germination rate of *Acokanthera oblongifolia* Hochst.

Figure 2: Effect of different treatments of sodium alginate and irradiated sodium alginate on germination percentage of *Acokanthera oblongifolia* Hochst.

Figure 3: Effect different treatments of sodium alginate and irradiated sodium alginate on relative height growth rate of *Acokanthera oblongifolia* Hochst during the season of (2015).
Figure 4: Effect different treatments of sodium alginate and irradiated sodium alginate on relative height growth rate of *Acokanthera oblongifolia* Hochst during the season of (2016).

Table 1: Means of plant height (cm), leaves number, plant fresh weight (gm.), plant dry weight (gm.), leave area (cm$^2$) and stem diameter (mm) of *Acokanthera oblongifolia* Hochst. as influenced by 50 ppm SA and various concentrations of ISA during the two seasons of 2015 and 2016.

<table>
<thead>
<tr>
<th>Treatments (ppm)</th>
<th>2015 Plant height (cm)</th>
<th>2016 Plant height (cm)</th>
<th>2015 Leaves number</th>
<th>2016 Leaves number</th>
<th>2015 Plant fresh weight (gm.)</th>
<th>2016 Plant fresh weight (gm.)</th>
<th>2015 Plant dry weight (gm.)</th>
<th>2016 Plant dry weight (gm.)</th>
<th>2015 Leave area (cm$^2$)</th>
<th>2016 Leave area (cm$^2$)</th>
<th>2015 Stem diameter (mm)</th>
<th>2016 Stem diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.81d</td>
<td>12.25c</td>
<td>10.00f</td>
<td>8.00f</td>
<td>4.07c</td>
<td>5.38d</td>
<td>1.72c</td>
<td>1.37d</td>
<td>8.75d</td>
<td>11.56c</td>
<td>1.64b</td>
<td>1.60bc</td>
</tr>
<tr>
<td>50 SA</td>
<td>14.86cd</td>
<td>15.17b</td>
<td>11.11edf</td>
<td>11.27ed</td>
<td>5.42bc</td>
<td>6.22cd</td>
<td>2.03c</td>
<td>1.93cd</td>
<td>11.23cd</td>
<td>15.00bc</td>
<td>1.76b</td>
<td>1.78bc</td>
</tr>
<tr>
<td>50 ISA</td>
<td>19.17ab</td>
<td>20.83a</td>
<td>13.83a</td>
<td>15.78a</td>
<td>7.42a</td>
<td>10.70a</td>
<td>3.48a</td>
<td>3.92a</td>
<td>14.37ab</td>
<td>24.14a</td>
<td>1.97a</td>
<td>2.29a</td>
</tr>
<tr>
<td>75 ISA</td>
<td>19.33a</td>
<td>21.92a</td>
<td>13.45ab</td>
<td>13.56b</td>
<td>7.46a</td>
<td>8.30b</td>
<td>3.47a</td>
<td>2.62b</td>
<td>16.00a</td>
<td>17.89b</td>
<td>2.01a</td>
<td>1.92b</td>
</tr>
<tr>
<td>100 ISA</td>
<td>18.08ab</td>
<td>20.83a</td>
<td>12.22bc</td>
<td>12.11bc</td>
<td>6.62ab</td>
<td>7.41bc</td>
<td>2.82bc</td>
<td>2.33bc</td>
<td>12.75bc</td>
<td>15.57bc</td>
<td>1.94a</td>
<td>1.91b</td>
</tr>
<tr>
<td>125 ISA</td>
<td>17.89ab</td>
<td>19.92a</td>
<td>11.78cd</td>
<td>10.33df</td>
<td>6.59ab</td>
<td>7.21bc</td>
<td>2.60b</td>
<td>2.10c</td>
<td>13.61abc</td>
<td>16.97bc</td>
<td>1.72b</td>
<td>1.89b</td>
</tr>
<tr>
<td>150 ISA</td>
<td>16.89bc</td>
<td>19.33a</td>
<td>10.44df</td>
<td>10.06df</td>
<td>5.64bc</td>
<td>6.99cd</td>
<td>2.12c</td>
<td>2.04c</td>
<td>12.66bc</td>
<td>16.27b</td>
<td>1.67b</td>
<td>1.84b</td>
</tr>
<tr>
<td>L.S.D. at 0.05</td>
<td>2.32</td>
<td>2.28</td>
<td>1.29</td>
<td>1.63</td>
<td>1.62</td>
<td>1.28</td>
<td>0.46</td>
<td>0.47</td>
<td>2.57</td>
<td>4.19</td>
<td>0.17</td>
<td>0.18</td>
</tr>
</tbody>
</table>

L.S.D. = Least significant different at 0.05 level of probability

3- Roots characteristics:

Data in Table (2) cleared that all ISA treatments caused a significant increase in roots volume and dry weight. Also, there was no significant difference between the spray of 50 ppm un-irradiated sodium alginate and control.

The highest increase in root volume was obtained by application of 50 ppm ISA by 147.71% and 111.11% compared to control in the first and second season respectively. Also foliar spray of 50 ppm ISA caused the heaviest root dry weight by 97.42% and 102.5% in both seasons respectively compared to control.

Table 2: Root volume (cm$^3$) and Root dry weight (g) of *Acokanthera oblongifolia* Hochst. as influenced by 50 ppm SA and various concentrations of ISA during the two seasons of 2015 and 2016.

<table>
<thead>
<tr>
<th>Treatments (ppm)</th>
<th>2015 Root volume (cm$^3$)</th>
<th>2016 Root volume (cm$^3$)</th>
<th>2015 Root dry weight (g)</th>
<th>2016 Root dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.50e</td>
<td>4.50e</td>
<td>2.33c</td>
<td>2.00e</td>
</tr>
<tr>
<td>50 SA</td>
<td>4.50de</td>
<td>6.00de</td>
<td>2.68bc</td>
<td>2.21de</td>
</tr>
<tr>
<td>50 ISA</td>
<td>8.67a</td>
<td>9.50a</td>
<td>4.60a</td>
<td>4.05a</td>
</tr>
<tr>
<td>75 ISA</td>
<td>7.83ab</td>
<td>8.50ab</td>
<td>4.30a</td>
<td>3.68ab</td>
</tr>
<tr>
<td>100 ISA</td>
<td>7.42abc</td>
<td>7.50bc</td>
<td>3.24b</td>
<td>3.27bc</td>
</tr>
<tr>
<td>125 ISA</td>
<td>6.33bcd</td>
<td>7.00cd</td>
<td>2.92bc</td>
<td>2.75cd</td>
</tr>
<tr>
<td>150 ISA</td>
<td>5.58cd</td>
<td>6.33cde</td>
<td>2.73bc</td>
<td>2.40de</td>
</tr>
<tr>
<td>L.S.D. at 0.05</td>
<td>1.96</td>
<td>1.31</td>
<td>0.86</td>
<td>0.67</td>
</tr>
</tbody>
</table>

L.S.D. = Least significant different at 0.05 level of probability
4- Chemical analysis.

Data in Table (3) showed that the highest increase in chlorophyll a and chlorophyll b content was obtained after foliar spray with 50 ppm ISA.

Application of ISA at 50 and 75 ppm in the first season caused the highest increase of chlorophyll a by 124.14% and the treatment 5 ppm ISA in the second season caused the highest increase by 152.9% compared to control plants. For chlorophyll b these treatments resulted in the same trend as 50 and 75 ppm of ISA caused the highest increase by 72.2% in the first season and the treatment of 50 ppm by 121.42% in the second season compared with control.

For total carbohydrate content, the highest significant increase was obtained by foliar spray of 50 ppm ISA by 60.39% and 50.9% compared with the control in the first and second season respectively. For Anthocyanin content, there was insignificant difference between treatments.

### DISCUSSION

Oligomers obtained from sodium alginate acted like endogenous growth elicitor and worked as signal molecules to trigger the synthesis of different enzymes and activate various plant responses, exploiting the gene expression as like the case of Cadmium stress (Ma et al., 2010) The ability of oligosaccharides to induce physiological processes in plants depends on the specific structure and size of it (Darvill et al., 1992).

Application of gel permeation chromatography (GPC) study revealed that there was a low molecular weight of fraction ISA (Naeem et al., 2012). Which may be the cause of its response on plant growth. Nevertheless, more investigations are needed to study how ISA stimulate plant growth.

The results of this study showed that application of foliar spray of ISA resulted in enhancement of all studied characters this results are in harmony with those obtained by Aftab et al., 2011 on Artemisia, Khan et al., (2011) on opium and Sarfaraz et al., 2011 on fennel and Naeem et al., 2012, 2014 on mint. Application of ISA caused a significant increase in leaf area which may be affect in gaining more sunlight to use extra CO<sub>2</sub> to increase photosynthesis and accumulation of more dry matter. There was a significant increase in root characteristics and shoot elongation which may be attributed to the growth promoting the effect of ISA (El-Rehim, 2006; Naeem et al., 2014).

### CONCLUSION

Soaking seeds of of *Acokanthera oblongifolia* Hochst in 50 ppm irradiated sodium alginate for 24 hours increase germination rate and percentage. Also irradiated sodium alginate can be used as a foliar spray on *Acokanthera* seedling at 50 ppm. This treatment caused an increase in vegetative growth parameters, root characteristics, chlorophyll content and total carbohydrate content. More studies are needed to know how ISA stimulate seed germination and plant growth.

### REFERENCES


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الملخص العربي

دراسة استخدام الصوديوم في إيجينات الصوديوم والحشوات

اسماء محمد طه، منى عبد الحميد سرور
فرع بحوث نباتات الزينة بطنطا- الاسكندرية
قسم بحوث الزينة وتنسيق الحدائق- معهد بحوث النباتات - مركز البحوث الزراعية

اجريت تجربتين في فرع بحوث نباتات الزينة بطنطا- الاسكندرية، معهد بحوث النباتات، وزارة الزراعة، الاسكندرية، جمهورية مصر العربية خلال موسمي (2015/2016) و(2016/2017) لدراسة تأثير الصوديوم في إيجينات الصوديوم والحشوات على نمو نباتات الكوكتلية. التحية الأولى دراسة تأثير الصوديوم في إيجينات الصوديوم والحشوات

Acokanthera oblongifolia Hochst

المشاع على نباتات بذور الكوكتلية في المعاملات الأثباتية (50 جوز في المليون من الصوديوم إيجينات والتركيزات الأثباتية من الصوديوم إيجينات المشاع (صفر، 50، 100 و150 جوز في المليون). وأظهرت النتائج أن الفرق لمدة 24 ساعة في محلول 50 و125 جوز في المليون من الصوديوم إيجينات المشاع ادى إلى زيادة في معدل ونسبة الأثبات، التحية الثانية دراسة تأثير الصوديوم في إيجينات الصوديوم إيجينات المشاع على نمو نباتات الكوكتلية حيث تم رش النباتات بنفس المعاملات المستخدمة في التحية الأولى واظهرت النتائج ان استخدام المعاملات الصوديوم إيجينات المشاع أدت إلى زيادة في اغلب الصفات المذروسة والمعاملة 50 جوز في المليون من الصوديوم إيجينات المشاع أدت إلى أعلى زيادة معنوية في كل الصفات الخضرية (ارتفاع النباتات، عدد الأوراق، الوزن الطازج والجاف للنبات، المساحة الورقية، قطر الساق) الصفات الجذرية (حجم الجذور والوزن الجاف للجذور) محتوى الأوراق من الكربوهيدرات وكمية الالنتن من الكربوهيدرات.