

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 solution 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 ,leave 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 $\text{NaC}_6\text{H}_7\text{O}_6$ 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 Mars 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):

Germination rate (GR) =

$$\frac{a+(a+b)+(a+b+c)+\dots+(a+b+c+m)}{n(a+b+c+m)}$$

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):

$$\text{Germination percentage (\%)} = \frac{\text{number of germinated seeds}}{\text{total seed number}} \times 100$$

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

$$\text{Relative height growth rate} = \frac{\ln H2 - \ln H1}{T2 - T1}$$

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.

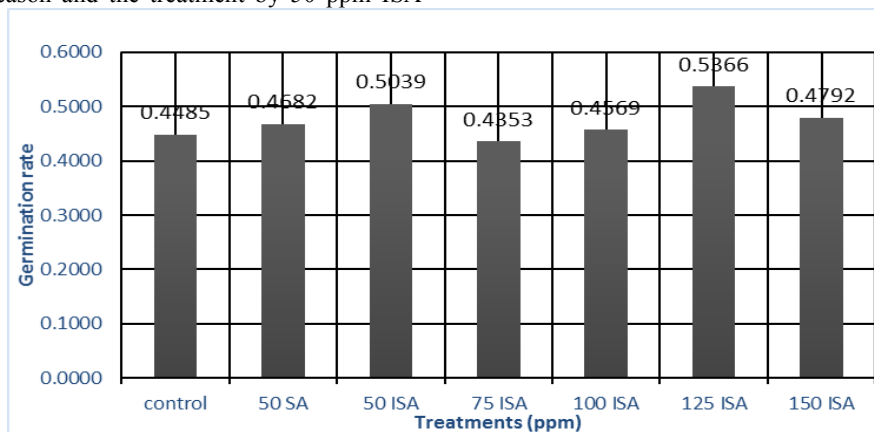


Figure 1: Effect of different treatments of sodium alginate and irradiated sodium alginate on germination rate of *Acokanthera oblongifolia* Hochst.

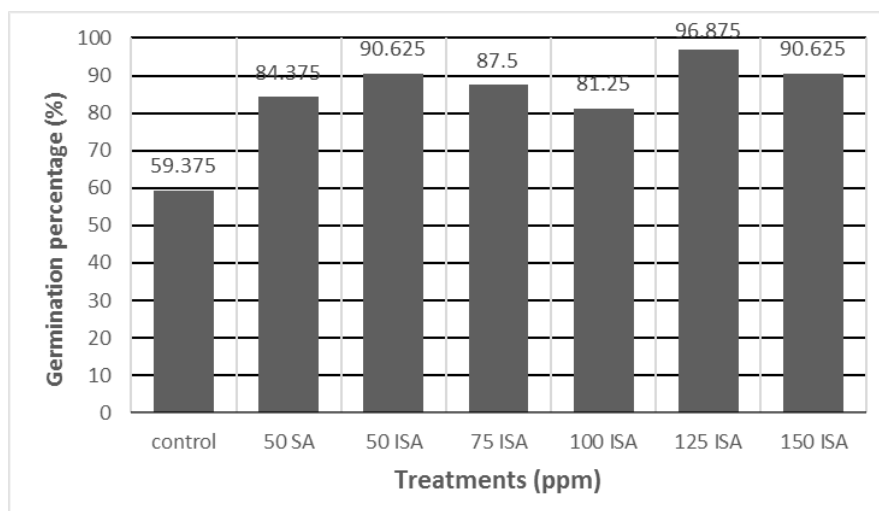


Figure 2: Effect of different treatments of sodium alginate and irradiated sodium alginate on germination percentage of *Acokanthera oblongifolia* Hochst.

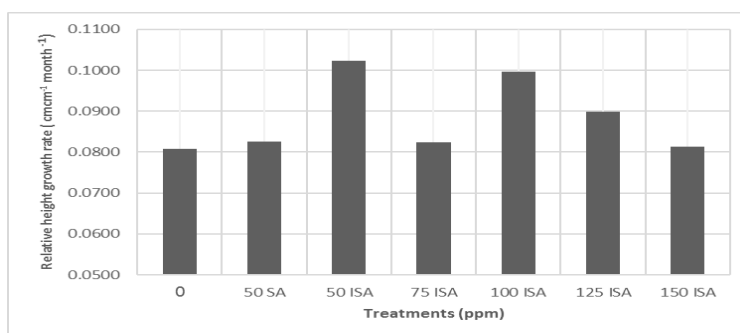


Figure 3: Effect different treatments of sodium alginate and irradiated sodium alginate on relative height growth rate of *Acokanthera oblongifolia* Hochst during the season of (2015).

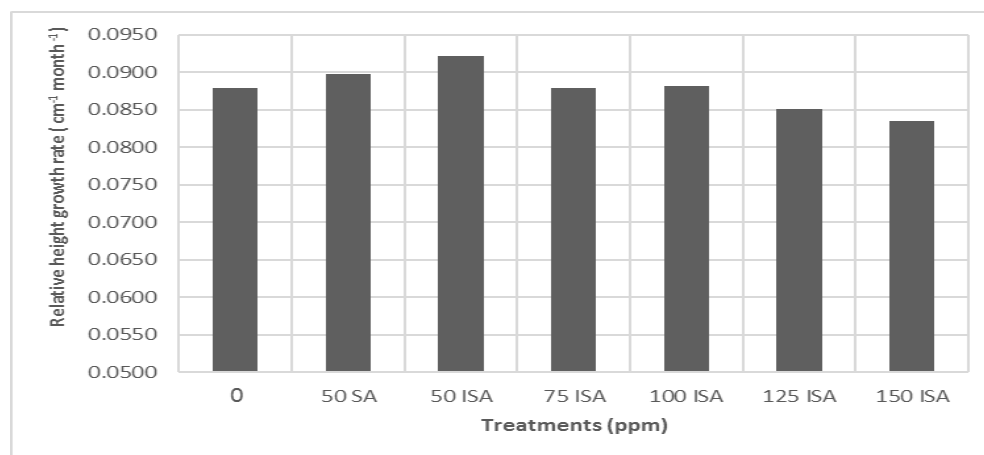


Figure 4: Effect different treatments of sodium alginate and irradiated sodium alginate on relative height growth rate of *Acokanthera oblongifolia* Hochst during the season of (2016).

Table 1: Means of plant height (cm), leaves number, plant fresh weight (gm.), plant dry weight (gm.), leave area (cm²) 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.

Treatments (ppm)	Plant height (cm)		Leaves number		Plant fresh weight (gm.)		Plant dry weight (gm.)		Leave area (cm ²)		Stem diameter (mm)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Control	12.81d	12.25c	10.00f	8.00f	4.07c	5.38d	1.72c	1.37d	8.75d	11.56c	1.64b	1.60bc
50 SA	14.86cd	15.17b	11.11cdf	11.27cd	5.42bc	6.22cd	2.03c	1.93cd	11.23cd	15.00bc	1.76b	1.78bc
50 ISA	19.17ab	20.83a	13.83a	15.78a	7.42a	10.70a	3.48a	3.92a	14.37ab	24.14a	1.97a	2.29a
75 ISA	19.33a	21.92a	13.45ab	13.56b	7.46a	8.30b	3.47a	2.62b	16.00a	17.89b	2.01a	1.92b
100 ISA	18.08ab	20.83a	12.22bc	12.11bc	6.62ab	7.41bc	2.82b	2.33bc	12.75bc	15.57bc	1.94a	1.91b
125 ISA	17.89ab	19.92a	11.78cd	10.33df	6.59ab	7.21bc	2.60b	2.10c	13.61abc	16.97b	1.72b	1.89b
150 ISA	16.89bc	19.33a	10.44df	10.06df	5.64bc	6.99cd	2.12c	2.04c	12.60bc	16.27b	1.67b	1.84b
L.S.D.at 0.05	2.32	2.28	1.29	1.63	1.62	1.28	0.46	0.47	2.57	4.19	0.17	0.18

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 (cm³) 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.

Treatments (ppm)	Root volume (cm ³)		Root dry weight (g)	
	2015	2016	2015	2016
Control	3.50e	4.50e	2.33c	2.00e
50 SA	4.50de	6.00de	2.68bc	2.21de
50 ISA	8.67a	9.50a	4.60a	4.05a
75 ISA	7.83ab	8.50ab	4.30a	3.68ab
100 ISA	7.42abc	7.50bc	3.24b	3.27bc
125 ISA	6.33bcd	7.00cd	2.92bc	2.70cd
150 ISA	5.58cd	6.33cde	2.73bc	2.40de
L.S.D.at 0.05	1.96	1.31	0.86	0.67

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

Table 3: Means of chlorophyll A (mg/g fresh weight), chlorophyll B (mg/g fresh weight), anthocyanin and total carbohydrate (%) of *Acokanthera oblongifolia* Hochst. as influenced by 50 ppm SA and various concentrations of ISA during the two seasons of 2015 and 2016.

Treatments (ppm)	Chlorophyll a (mg/g fresh weight)		Chlorophyll b (mg/g fresh weight)		Total Carbohydrate (%)		Anthocyanin Content	
	2015	2016	2015	2016	2015	2016	2015	2016
Control	0.29b	0.34d	0.11c	0.14d	5.58c	6.01c	0.088	0.113
50 SA	0.31b	0.50c	0.14b	0.18cd	6.71bc	6.71bc	0.085	0.108
50 ISA	0.65a	0.86a	0.19a	0.31a	8.95a	9.07a	0.078	0.088
75 ISA	0.65a	0.68b	0.19a	0.28ab	8.06ab	8.71a	0.069	0.071
100 ISA	0.60a	0.68b	0.18a	0.27ab	7.76ab	7.77ab	0.083	0.069
125 ISA	0.57a	0.68b	0.16a	0.23bc	7.72ab	7.75abc	0.078	0.072
150 ISA	0.56a	0.67b	0.16a	0.16cd	6.52bc	7.68abc	0.085	0.078
L.S.D.at .05	0.11	0.09	0.04	0.07	1.52	1.75	N.S.	N.S.

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 50 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 CO₂ 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 *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.

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الملخص العربي

دراسة استخدام الصوديوم إيجينات والصوديوم إيجينات المشع على نمو نباتات الكوكتا

اسماء محمد طه، منى عبد الحميد سرور

فرع بحوث نباتات الزينة بأنطونيداس - الإسكندرية

قسم بحوث الزينة وتنسيق الحدائق - معهد بحوث البساتين - مركز البحوث الزراعية

اجريت تجربتين في فرع بحوث نباتات الزينة بأنطونيداس، معهد بحوث البساتين، وزارة الزراعة، الاسكندرية، جمهورية مصر العربية خلال موسمي (٢٠١٤/٢٠١٥)، (٢٠١٥/٢٠١٦) لدراسة تأثير الصوديوم إيجينات والصوديوم إيجينات المشع على إنبات ونمو نباتات الكوكتا *Acokanthera oblongifolia* Hochst. التجربة الأولى دراسة تأثير الصوديوم إيجينات والصوديوم إيجينات المشع على إنبات بذور الكوكتا في المعاملات الأتية (٥٠ جزء في المليون من الصوديوم إيجينات والتركيزات الأتية من الصوديوم إيجينات المشع (صفر، ٥٠، ٧٥، ١٠٠، ١٢٥ و ١٥٠ جزء في المليون). وظهرت النتائج أن النقع لمدة ٢٤ ساعة في محلول ٥٠ و ١٢٥ جزء في المليون من الصوديوم إيجينات المشع ادي إلى اعلى زيادة في معدل ونسبة الأنبات. التجربة الثانية دراسة تأثير الصوديوم إيجينات والصوديوم إيجينات المشع على نمو نباتات الكوكتا حيث تم رش النباتات بنفس المعاملات المستخدمة في التجربة الأولى و اظهرت النتائج ان استخدام معاملات الصوديوم إيجينات المشع ادت إلى زيادة في اغلب الصفات المدروسة والمعاملة ٥٠ جزء في المليون من الصوديوم إيجينات المشع أدت إلى أعلى زيادة معنوية في كل الصفات الخضرية (ارتفاع النبات، عدد الأوراق، الوزن الطازج والجاف للنبات، المساحة الورقية، قطر الساق) الصفات الجذرية (حجم الجذور والوزن الجاف للجذور)، محتوى الاوراق من الكلوروفيل ومحتوى النبات من الكربوهيدرات.