

**EFFECT OF SODIUM AZIDE ON GROWTH AND FLOWERING OF  
 BOUGAINVILLEA GLABRA CHOICY VAR SANDERIANA  
 BY**

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**ABSTRACT**

A series of pot experiment was conducted at the Nursery of Hort. Res. Inst., ARC., Giza, Egypt during 2004/2005 and 2005/2006 seasons to find out the effect of sodium azide added with either a soil drench or a foliar spray, twice with one month interval, with the concentrations at 0.00, 0.02, 0.04 and 0.08% on growth, flowering and chemical composition of *Bougainvillea glabra choicy* var. *sanderiana* transplants growth in 16-cm-diameter plastic pots filled with about 1.5 Kg of an equal mixture of loam, sand and peatmoss, by volume.

The obtained results indicated that plant height (cm) and No. leaves/plant were progressively decreased with increasing sodium azide concentration, whereas stem diameter (cm), No. branches/plant and leaf area (cm<sup>2</sup>) were gradually increased with significant differences in most cases of the two seasons. Sodium azide at 0.02% level significantly raised No. peduncles/plant, while both 0.02 and 0.04% levels greatly evaluated No. Florets /peduncle. On the contrary peduncle length (cm) was cumulatively declined as sodium azide concentration increased.

A significant increment in the leaves content of chlorophyll a,b and carotenoids was observed due to the low (0.02%) and medium (0.04%) rates, but they were markedly decreased in response to the high rate (0.08 %). The opposite was the right regarding total indoles and total phenols, as they were argumentatively depressed with increasing sodium azide level.

Concerning application method, data showed that soil drench technique caused a significant improve in plant height, No. branches /plant and the content of pigments in the leaves, while foliar spray method significantly improved No. leaves/plant, peduncle length and the content of both total indoles and total phenols. Application method, however had no significant effect on stem diameter, leaf area, No. peduncles/plant and No. florets/peduncle parameters.

From the previous results, it could be concluded that drenching or spraying *B. glabra* var. *sanderiana* transplants with 0.02 or 0.04% sodium azide aqueous solution, twice with one month interval gave compact and floriferous plants suitable for marketing as flowering pot plants. Moreover, the occurrence of some reverse responses may suggest the incidence of some changes in the response pattern (such as increasing of branching, leaf area and number of both peduncles and florets) which may lead to some variations in the gene expression.

**Key words:** *Bougainvillea*, sodium azide, mutations, soil drench, foliar spray, vegetative growth, flowering, chemical composition.

**INTRODUCTION**

*Bougainvillea* of the nyctaginaceae family is a south american genus comprising 18 species of tropical and subtropical woody

vines or scandent shrubs. Most varieties flower continuously and used to drape walls, pillars buildings and bare-stemmed trees, or

hardly cut back to be flowering hedges or large shrubs (Bailey, 1976). Among the widest spreading species is *B. glabra* choicy var. *sanderiana*. It is a very floriferous and blooming even in very small pots. Also it is a very worthy plant both for pot culture or as a shrub or climber. Often blooms when 1 Ft. height, but reaches a height of 10-12 fts. (Huxley *et al.*, 1992). On the other hand, mutations can produce a wide range of genetic variations, which may result in desirable characters. These mutations may be occurred naturally or physically by irradiation or chemically by chemical mutagens. Sodium azide ( $\text{NaN}_3$ ) is one of the most important as a effective chemical mutagens. It is well known as the chemical found in automobile airbags, (Owais *et al.*, 1981), however produced a high number of mutations in many plant species.

In this concern, Kashikar and Khalatkar (1980) found that exposed seeds of *Petunia hybrida* to sodium azide at 0.005% for 18 h induced mutations in the genes controlling anthocyanin synthesis, affecting venation in various parts of the flower.

Padmaja and Sudhakar (1987) isolated 6 floral mutants from *Petunia* axillaries populations raised from seeds treated with

sodium azide at 0.025% for 12 h. Wang-Ping *et al.* (1996) noticed that germination and emergence percentages and seedling height of 5 sunflower (*Helianthus annuus*) cultivars were decreased when soaked in 0.007 M of  $\text{NaN}_3$  for 6 h. Likewise, Bhate (1999) mentioned that different concentrations of sodium azide induced useful mutations accompanied with more flower number in violet blue and red purple genotypes of *Ipomoea purpurea*. Similar results were also gained by Youssef *et al.* (1998) on *Melaleuca armillaris*, Vainola (2000) on *Rhododendron* hybrid Youssef (2003) on *Yucca elephantipes* and *Philodendron scandens* and Youssef and Abou-Dahab (2006) on *Populus alba*. Also, Sayed *et al.* (2007) indicated that subjecting the explants of *Solidago altissima* var. "tara" to 0.05 mg/L of sodium azide for 10, 20 or 40 min gave the greatest number of leaves. It was also produced the highest root response (100%). Sodium azide had less effect on improving pigments, indoles and phenols content. This trial, however aims to find out the response of *Bougainvillea glabra* choicy. Var. *sanderiana* transplants to the different concentrations of sodium azide when applied either as a soil drench or as a foliar spray.

## MATERIALS AND METHODS

A study was consummated at the Nursery of Hort. Res. Inst., ARC, Giza, Egypt throughout the two successive seasons of 2004/2005 and 2005/2006 to study the effect of sodium azide at various levels, two application method and their interaction on growth, flowering and chemical composition of *Bougainvillea glabra* choicy. var. *sanderiana*, so, cuttings of *sanderiana* with initial long of 12-15 cm and 1-1.2 cm diameter, were taken from the middle parts of woody branches of five- years-old shrub and dipped in a fungicide solution of 0.2% Topsin M-70% WP (produced by Sumitomo) chemical Co., Ltd., Osaka, Japan) for 15 minutes. Then, they were planted on July, 26 for both seasons in 8-cm-diameter plastic pots (one cutting/pot) filled with an equal mixture of loam, sand and peatmoss (1:1:1, by volume). All cuttings were successfully rooted in the proper time. After

rooting by two months, the rooted cuttings were transplanted into 16-cm-diameter plastic pots filled with 1.5 Kg of the same previous mixture. On December, 26<sup>th</sup>, pots were divided into two main groups, the first was treated with sodium azide as a soil drench, while the second treated with the same chemical as a foliar spray. Each main group, however was divided into four sub-groups to receive the different concentrations of the aqueous solution of sodium azide, which were: 0.00% (referred to as control) 0.02%, 0.04% and 0.08%, referred to as low medium and high levels respectively). Application was repeated once again after one month later. The layout of such factorial experiment was a completely randomized design (Das and Giri, 1986) consisting of 8 treatments, as each treatment included 15 plantlets in 3 replicate. At the end of experiment (on 26<sup>th</sup> of

stumper),the following data were recorded: plant height (cm),stem diameter at the base (cm), number of branches and leaves,plant, leaf area(cm<sup>2</sup>) number of peduncles//plant, peduncles length number of florets/peduncle.

In fresh leaf samples, however chlorophyll a,b and carotenoids (mg/g F.W.) according to the method of Moran (1982),as

well as total indoles (Selim *et al.* ,1978) and total phenols (Daniel and George,1972)were determined in the second season only .

Data were then statistically analyzed according to SAS program (1994)using Duncan,s Multiple range test to verify the significance level among means of various treatments described by (Duncan,1955).

## RESULTS AND DISCUSSION

**Effect of sodium azide level, application method and their interaction on:**

### 1.Vegetative growth:

From data averaged in Table (1), it is clear that plant height (cm) and number of leaves/plant were progressively decreased with increasing sodium azide concentration in the two seasons to reach the minimum values for the high level (0.08%), which gave the least means in this concern. The opposite was the right regarding stem diameter (cm), No. branches/ plant and leaf area (cm<sup>2</sup>) as they were gradually increased as a result of elevating sodium azide concentration with significant differences in most cases of both seasons. As for application method, data reveal that applying the mutagen as a soil drench induced a significant increment in plant height and number of branches/plant, but significantly declined No. leaves/plant in the two seasons.

So, the best height and branching were found due to soil drench technique, while for number of leaves trait, foliar spray method was the best .Application method, however had no significant effect on stem diameter and leaf area characters in both seasons .

Generally, the best appearance of Bougainvillea plants obtained from drenching sodium azide solution at the low and medium concentrations (0.02 and 0.04 %, respectively), as these two levels gave the suitable balance between plant height and number of both branches and leaves/plant.

Reduction of plant height and No. leaves may be attributed to the high toxicity of sodium azide and to its direct role on

depression the biosynthesis of some amino acids, such as L- cysteine and O- acetylserine, in the meristematic cells (Owais *et al.*, 1981), while increasing No. branches may refer to the ability of such chemical mutagen to overcome the apical dominance and hence,stimulate the lateral buds to develop into new shoots (Vainola 2000).

These results, however are in accordance with those attained by Wang-Ping *et al.* (1996) on five cvs. of sunflower (*Helianthus annus*) Youssef *et al.* (1998) on *Melaleuca armillaris* and Youssef and Abou-Dahab (2006) on *Populus alba*.

### 2- Flowering:

Data in Table (2) exhibit that sodium azide at 0.02% level significantly elevated No. beduncles/plant over control and other levels in the two seasons,while peduncle length (cm) was augmentatively declined with increasing sodium azide concentration, except for the low one (0.02%), which gave means closely near to those of control plants in both seasons when applied as foliar spray.

AS for No. florets/peduncle, data showed that it was greatly increased in response to both low (0.02%) and medium (0.04%) concentrations with significant differences compared to the means of control and high concentration (0.08%)in the two seasons . Regarding the effect of application method, it is obvious that such factor had no a significantly effect on flowering characters measured in this work, with the exception of foliar spray technique that significantly improved peduncle length (cm)in both seasons comparing with soil drench method.

Table (1): Effect of sodium azide treatments, application methods and their interaction on some vegetative growth parameters of *Bougainvillea glabra* Choisy var *Sanderiana* transplants during 2004/2005 and 2005/2006 seasons.

sodium azide treatments	Plant height (cm)			Stem diameter (cm)			No. branches/plant			No. leaves/plant			Leaf area (cm <sup>2</sup> )		
	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean
<b>First season: 2004/2005</b>															
0.00%	36.17a	35.00a	35.59a	1.36cb	1.33c	1.35b	2.00cb	1.86c	1.93c	21.67ab	23.00a	22.34a	8.00d	8.00c	8.00d
0.02%	32.67ba	30.76b	31.72b	1.48ba	1.45b	1.47b	2.33b	2.10cb	2.22b	18.33cb	19.68b	19.01b	11.42c	15.50a	13.46c
0.04%	31.26b	28.33c	29.80bc	1.56ab	1.51ba	1.54ba	2.98a	2.38b	2.68ba	13.60d	16.00c	14.80c	11.67b	9.17b	10.42b
0.08%	329.33c	24.48d	26.91c	1.67a	1.60a	1.64a	3.11a	2.89ab	3.00a	14.00dc	14.79cd	14.40c	12.17a	9.17b	10.67a
Mean	32.36a	29.64b		1.52a	1.47a		2.61a	2.31b		16.90b	18.37a		10.81a	10.46b	
<b>Second season: 2005/2006</b>															
0.00%	47.00a	39.51ba	43.26a	1.13cb	1.08c	1.11b	2.00cb	1.84c	1.92c	19.99ab	21.56a	20.78a	8.78d	8.50d	8.64d
0.02%	36.28b	35.64b	35.64b	1.35ab	1.17b	1.26ba	2.31bc	2.12cb	2.22b	17.38b	19.00ba	18.19b	10.33b	9.33c	9.83c
0.04%	35.10b	31.98cb	31.98cb	1.41a	1.33ba	1.37a	2.76ba	2.43b	2.60ba	13.76c	12.85dc	13.31c	12.03ba	12.10ba	12.07b
0.08%	29.33cb	27.16c	28.25c	1.48a	1.42a	1.45a	3.00a	2.78ba	2.89a	12.33d	13.00cd	12.67d	12.86ab	13.58a	13.22a
Mean	36.93a	32.63b		1.34a	1.25a		2.52a	2.29b		15.87b	16.60a		11.00a	10.88a	

Table (2): Effect of sodium azide concentrations, application methods and their interaction on flowering of *Bougainvillea glabra* choicy var sanderiana transplants during 2004/2005 and 2005/2006 seasons.

Sodium azide concentrations	No. peduncles/plant			Peduncle length (cm)			No. florets/peduncle		
	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean
<b>First season: 2004/2005</b>									
0.00%	1.00 b	1.00 b	1.00 b	19.33 b	22.36 a	20.85 a	3.33 b	3.51 b	3.42 b
0.02%	1.87 a	2.00 a	1.94 a	17.67 c	21.63 a	19.65 b	4.67 a	5.00 a	4.84 a
0.04%	1.28 ba	1.00 b	1.00 b	17.00 c	19.67 b	18.34 c	4.67 a	4.68 a	4.368 a
0.08%	1.00 b	1.00 b	1.00 b	16.28 d	18.26 cb	17.27 d	3.00 b	2.67 c	2.84 c
Mean	1.22 a	1.25 a		17.57 b	20.48 a		3.92 a	3.97 a	
<b>Second season: 2005/2006</b>									
0.00%	1.00 b	1.00 b	1.00 b	19.16 ba	21.96 a	20.56 a	4.00 b	4.00 b	4.00 b
0.02%	2.00 a	3.00 a	2.50 a	13.43 b	21.00 a	19.72 ab	5.33 a	6.33 a	5.83 a
0.04%	1.33 ba	1.00 b	1.00 b	17.78 cb	19.00 ba	18.39 b	5.21 a	5.00 b	5.11 a
0.08%	1.00 b	1.00 b	1.17 b	13.33 d	15.33 c	14.33 c	4.00 b	3.88 c	3.94 c
Mean	1.33 a	1.50 a		17.18 b	19.32 a		4.64 a	4.80 a	

In general, the prevalence in most flowering parameters in the two seasons was due to the low and medium levels of sodium azide (0.02 and 0.04%, respectively) applied either as a soil drench or as a foliar spray, with the superiority of the latter in some cases.

The promotive effect of the low and medium concentrations of sodium azide may be due to the direct action of this chemical mutagen under these concentrations on flowering induction process in the apex and its ability to prevent the emanation of some positive inhibitory influences from the leaves under non-inductive conditions as noticed by Padmaja and Sudhakar (1987) on *Petunia axillaris* and Bhate (1999) on *Ipomoea purpurea*, as they reported that sodium azide at low level increased number of flowers / plant. Moreover, Sayed *et al.* (2007) postulated that sodium azide at 0.05 mg/l gave the greatest number of shootlets, and consequently more number of flowers in *Solidago altissima* var. "tara" explant.

Although there was a reduction in plant height and number of leaves in Bougainvillea transplants exposed to the low and medium rates of sodium azide, the corresponding increase in branching, leaf area and

number of / peduncles / plant, which was coupled with increasing number of florets/peduncle improved performance of the resulted plants causing them more suitable for marketing as flowering pot plants.

**3-Chemical composition:**

Concerning photosynthetic pigments content, as mg/g F.W. of leaves, data presented in Table (3) indicate that chlorophyll a,b, and carotenoids were significantly increased as a result of using low and medium levels, whereas the highest one (0.08%) greatly decreased them with significant differences in comparison with either low and medium levels or control averages.

Applying sodium azide as soil drench surpassed application of it as foliar spray. On the contrary, were those results of total indoles and total phenols content (mg/g F.W.), as they were cumulatively decreased with raising sodium azide concentration, with the mastery of foliar spray method on soil drench technique. The simultaneous depression of both total indoles and total phenols made the ratio between them constant irrespective of their concentrations in the tissues. Increasing pigments content by the low and medium rates of sodium azide may ascribed to the promotive

effect of such mutagen at these rates on stroma lamella formation, and grana and chlorophyll appearance during the normal leaf growth, while decrement this content by the high rate may attributed to the high toxicity of sodium azide. On the same line were those

results of Kashikar and Khalatkar(1980)on *Petunia hybrida*, Singh *et al.* (2001)on rice and Solanki and Phogat(2005)who mentioned that sodium azide at 0.02 and 0.04%induced chlorophyll mutations in lentil (*lens culinaris*).

Table (3): Effect of sodium azide concentrations, application methods and their interaction on some constituents of *Bougainvillea glabra* choicy var sanderiana transplants during 2004/2005 and 2005/2006 seasons.

Sodium azide concentrations	Chlorophyll a (mg/g. F.W)			Chlorophyll b (mg/g. F.W)			Carotenoids (mg/g. F.W)			Total indoles (mg/g. F.W)			Total phenols (mg/g. F.W)		
	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean	Soil drench	Foliar spray	Mean
0.00%	0.41 c	0.38 c	0.40 c	0.12 d	0.11 d	0.12 c	0.37 c	0.34 c	0.36	1.53 b	1.68 a	1.61 a	5.64 ba	6.03 a	5.84 a
0.02%	0.60 b	0.54 b	0.57 b	0.33 a	0.30 b	0.32 a	0.44 b	0.41 b	0.43	1.30 d	1.41 c	1.36 b	4.95 cb	5.35 b	5.15 b
0.04%	0.72 a	0.63 b	0.68 a	0.35 a	0.28 b	0.32 a	0.59	0.52 a	0.56	1.28 ed	1.33 d	1.31 b	4.62 d	4.88 c	4.75 c
0.08%	0.29 d	0.27 d	0.28 d	0.18 c	0.19 c	0.19 b	0.21 d	0.18 e	0.20	1.09 e	1.18 e	1.14 c	4.27 e	4.61 d	4.44 c
Mean	0.51 a	0.46 b		0.25 a	0.22 b		0.40 a	0.36 b		1.30 b	1.40 a		4.87 b	5.22 a	

Briefly, drenching or spraying *Bougainvillea glabra* choicy. var. sanderiana transplants with 0.02 or 0.04%of sodium azide aqueous solution, twice with one month interval, can be recommended to get compact and floriferous plants suitable from the commercial point of view for marketing as flowering pot plants.

However, unexpected responses that were not parallel with the the normal responses may suggest that some changes in the response pattern may have taken place. This means, in turn, that some variations in the gene expression could be inferred. So, the second part of this study will be devoted for proving this expectation.

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### تأثير المعاملة بالصوديوم آزيد على نمو وأزهارا لجهنمية

هنان محمد احمد يوسف ، فيصل محمد سعداوى

قسم بحوث نباتات الزينة-معهد بحوث البساتين -مركز البحوث الزراعية -الجيزة-مصر

اجريت سلسلة من تجارب الإصص بمشغل معهد بحوث البساتين ، مركز البحوث الزراعية، الجيزة، مصر خلال موسمي ٢٠٠٤/٢٠٠٥، ٢٠٠٥/٢٠٠٦ لدراسة تأثير إضافة المحلول المائي للصوديوم آزيد (مطر كيميائي) أما للتربة أو رشا على الأوراق، مرتين وبفاصل شهر بينهما، بتركيزات: ٠.٠٢، ٠.٠٤، ٠.٠٨ % على النمو والإزهار والتركيب الكيميائي لثلاث الجهنمية *Bougainvillea glabra* صنف سانديريانا النامية في إصص بلاستيك قطرها ١٦ سم ملأت بحوالي ١.٥ كجم من مخلوط متساوي من الطمي ، الرمل والبيتموس (١١١١ حجما) ولقد أوضحت النتائج المتحصل عليها؛ أن طول النبات وعدد الأوراق /نبات قد انخفضا تصاعدا بزيادة تركيز الصوديوم آزيد ، بينما زاد قطر الساق وعدد الأفرع /نبات ومساحة الورقة تدريجيا وبفروق معنوية في معظم الحالات لكلا الموسمين. ولقد أحدث التركيب المنخفض من الصوديوم آزيد (٠.٠٢ %) زيادة معنوية في عدد العنايد الزهرية /نبات بينما أدى كلا من التركيبين المنخفض (٠.٠٢%) والمتوسط (٠.٠٤%) من هذا المطر الى حدوث زيادة كبيرة في عدد الزهيرات /عقلود زهرى - على العكس فإن طول العقلود الزهرى قد انخفض تدريجيا كلما زاد تركيز الصوديوم آزيد - أيضا ' أحداث التركيبين المنخفض والمتوسط زيادة معنوية لى محتوى الأوراق من كلوروفيللى اىب والكاروتينويدات بينما أنخفض محتوى هذه الصبغات بدرجة ملحوظة استجابة للتركيز المرتفع (٠.٠٨ %). ولقد كان العكس صحيحا، فيما يتعلق بمحتوى الأوراق من الاندولات الكلية والفينولات الكلية، حيث انخفضا بشكل تدريجى بزيادة تركيز الصوديوم آزيد.

فيما يتعلق بطريقة الإضافة، فقد أوضحت النتائج أن إضافة المحلول للتربة أحدثت تحسنا ' معنويا' في ارتفاع النبات ، عدد الأفرع/نبات وكذلك محتوى الأوراق من الصبغات، بينما أحدثت إضافة المحلول

رشا "على الاوراق تحسنا معنويا" فى عدد الاوراق/نبات ،طول العنقود الزهرى ومحتوى الاوراق من الاندولات الكلية والفينولات الكلية. هذا، ولم يكن لطريقة الاضافة اى تأثير معنوى على صفات قطر الساق ،مساحة الورقة،عدد العناقيد الزهرية /نبات وعدد الزهيرات/عنقود زهرى.  
من النتائج السابقة، يمكن القول بان رى أو رش شتلات الجهنمية صنف (سانديريانا) بالمحلول المائى للصوديوم ازيد (أما بتركيز ٠٠٠٢ أو ٠٠٠٤ %) مرتين وبفاصل شهر بينهما، أعطى نباتات مندمجة غزيرة الإزهار تصلح للتسويق كنباتات أصص مزهرة. علاوة على ذلك،فإن حدوث بعض التأثيرات الغير متوقعة لهذا المطفر قد يوحى بحدوث بعض التغيرات فى نمط أو طريقة الاستجابة، والتي قد تؤدى إلى بعض التغيرات فى التعبير الجينى.