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Original Article

Effects of naphthalene acetic acid on aborted okra production, antioxidants, minerals, phenol, flavonoid, and carotenoid content applied to flower ovary stigma

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Abstract

The experiment was carried out to investigate the effect of different concentrations of NAA (naphthalene acetic acid) on the seedless (aborted) okra production, vitamin C, carotenoids, flavonoids, antioxidants (DPPH), phenolic, and mineral content. The micro-syringe injection in flower stigma was an innovative application method used in this experiment, rather than spray, which was a common and traditional method. The flower stigma injection method was applied on the flower stigma after the anthesis of the flower of the okra plant using NAA at different concentrations. In Experiment 1: The lowest concentration (25 mg/l) of NAA greatly increased the pod setting compared to the higher concentrations and control. NAA application at 25 and 50 mg/l concentrations induced higher values of pod length, diameter, size, weight, ascorbic acid, and soluble solid content over the control. The chlorophyll content in leaves was affected significantly by different concentrations of NAA. It was found that 25 and 50 mg/l concentrations of NAA significantly increased chlorophyll content, fiber, moisture, flavonoid, carotenoid, antioxidant (DPPH), minerals, and phenolic content compared to the other concentrations and control. In addition to that, control and 25 mg/l concentrations of NAA increased the production of healthy seeds compared to the 50 and 100 mg/l. Moreover, 50 and 100mg/l of concentrations showed higher aborted seed (seedless) than the other concentration and control. In Experiment 2: In the second year, the residual effects of aborted seed (seedless) were found to have a decreasing trend of most of the parameters like pod weight, size, aborted okra percent, leaf chlorophyll, antioxidant (DPPH), and Vitamin C. But, NAA concentrations showed better residual effects in the second year in comparison to the control. Therefore, it seemed that 25 mg/l was the best concentration for pod growth and development, and 100 mg/l was the best for seedless okra production in the first and second years.

Keywords: Aborted okra, Chlorophyll, Mineral, Flavonoid, Carotenoid, Antioxidant

1. Introduction

Okra has medicinal value and health benefits. Okra *(Abelmoschus esculentus*) is one of the important and popular vegetable crops in tropical and subtropical areas in Asia [1] and Africa [2]. Okra is also known as lady's finger, gumbo, bhindi in Malaysia, and bamya in Arabic countries. It belongs to the family Malvaceae, genus, *Abelmoschus and species, esculentus.* Cultivated okra is suitable for agriculture as a garden crop as well as on commercial farms. It is one of the vegetables grown commercially in many countries such as India, Western Africa, Iraq, the United States, and other countries around the world [3]. It was reported that the total production of okra was 5.9 m tons in the world [4]. The production was 1, 1.25, 1.26, 2.4, 8.1, and 38.5 m tons in the USA, Ivory Coast, Iraq, Sudan, Nigeria, and India, respectively [5].

It was mentioned [6] that okra pods are considered nutritious, providing some human supplementary vitamins such as vitamin C, A, B- complex, calcium, potassium, [7] iron, and other minerals. Okra pods contain many nutritional contents which important for human health. One hundred grams of the fresh pod has moisture (89.6%), K (103 mg), Ca (90 mg), Mg (43 mg), P (56 mg), vitamin C (18 mg), and some important metals such as iron and aluminum [7, 8].

The application of plant growth regulators is known as one of the most important treatments used nowadays in agriculture. The application of different growth regulators increased some horticulture crop production [9, 10]. Growth regulators mainly regulate the plant's physiological and biochemical processes. For example, they play a major role in dormancy, organ size, crop improvement, flowering, and fruit set, and regulation of the chemical composition of plants [11]. The phytohormone auxin af-

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fects approximately all developmental processes in plants, including fruit improvement. However, auxin is produced in meristems and young leaves and moved to other parts of the plant in a polar fashion [12].

There are more than 100 distinct gibberellins produced primarily in roots & young leaves but GA3 or gibberellic acid is the most popular available form. GA3 has many effects on plant growth such as enhanced stem and internode elongation, seed germination, enzyme production during germination fruit setting and growth [13, 14] and breaking of dormancy. It was stated that plant growth regulators may be used to regulate the vegetative growth of plants [15]. Application of GA3 increased the plant height, number of internodes, leaf area, dry weight of shoot, and dry weight of Gram plant, respectively [16,17]. However, research has been done on the use of plant growth hormone to improve vegetative growth and pod size and delay pod maturity in vegetables using the spray method. However, no studies have been conducted to evaluate the complete profile of vegetative growth, pod quality, and seed yield in response to NAA application to okra using innovative flower ovary stigma injection. Objectives of the study were undertaken to

To investigate the effect of different concentrations of NAA on seedless okra production, vitamin C, carotenoids, flavonoids, antioxidants (DPPH), phenolic, and mineral content Also to evaluate the efficacy of the flower stigma injection method application on seeds for inducing seedless (aborted seeds) in okra pod in the first year and the residual effects in the following (2^{nd}) year.

2. Materials and Methods

2.1. Study site and Climatic information

The experiment was carried out at the University of Malaya Experimental Farm and Garden, Malaysia, and Hail University Biology Central Laboratory, Hail, KSA. The area of this study had hot, humid tropical weather and the soil was peat, with a mean pH of 6.6.

2.1.1. Experiment 1: Plant materials and seed cultural operation

The seeds of the local *Abelmoschus esculentus* variety were sown in the experimental area of Banting. These seeds were soaked in distilled water for 24 hours and spread on moist filter paper. Okra seeds were sown directly into the soil by hand in soil fertilized with NPK 19 g/hill 14-14-14 as basal fertilization. Thirty days after emergence, side-dress with 10g/hill 46-0-0 (16) and plots were irrigated when necessary. The experiment was laid out in a randomized block design having four replications. The seeds were shown in rows made by hand plow. When germination was completed, thinning was done to maintain the plant-plant distance of 30 cm. The depth of planting was 1cm from the surface of the soil. Hoeing, weeding, and other cultural practices were done uniformly (Fig. 1).

2.1.2. Preparation of plant growth regulators

The growth regulators employed in the experiment were NAA. The concentrations of the growth regulator treatments were 25, 50, 100, and 200 mg/L. The NAA was dissolved in 2 ml of 1% ethanol to make the desired concentration. Each rate of chemical NAA was added with distilled water to make 100 ml of solutions. The control plants were injected with 100 ml of water mixed with 2 ml of 1% ethanol.

A B

Fig. 1. The flower stigma injection technique of okra. A. Okra flower ovary sigma injection by micro syringe B. Control (No injection).

2.1.3. Application

One and a half ml (1.5ml) of the various concentrations of NAA (0, 25, 50, 100, and 200 mg/l) were applied on the flower stigma (parts of the ovary) by injecting with a needle for a surgical purpose of 1 dose. While the control was distilled water mixed with 2ml of 1% ethanol (Fig. 1).

2.1.4. Measurement of parameters

Data were recorded considering the following parameters:

Leaf chlorophyll content: The chlorophyll content in the leaves was measured by the SPAD value meter (Minolta Japan).

2.1.4.1. Pod parameters

Five pods were randomly chosen from each treatment to determine the following characteristics: Green pod length (cm), green pod diameter (cm), and pod size (cm^2). Pod size was determined by measuring the length and diameter of the pod per treatment with a Varnier caliper.

2.1.4.2. Pod weight

Green pod weight (g) was determined with the help of a digital UWE-ESP Digital Electric Balance, and the average weight was calculated.

2.1.4.3. Seed production

For the determination of healthy seeds from treated flowers, the number of healthy seeds and aborted seeds were counted after the dry stage, healthy seeds/pod (%), and seedless or aborted seeds/pod (%).

2.1.4.4. Fiber and moisture determination

Fiber and moisture were determined from the pod sample according to the methods by the United Nations Economic Commission for Europe [17].

2.1.4.5. Flavonoid and Carotenoid determination

Total Flavonoid (FC) was determined using the methods described by aluminum chloride colorimetric assay, using catechin as a standard, and carotenoid was determined according to the methods by Lucia *et al* [18].

2.1.4.6. Determination of Antioxidant

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacities of the okra pod were determined based on the standard method [18]. Trolox was used as

the standard, and the DPPH radical scavenging capacities were presented as µmol of Trolox equivalent per gm of okra fruit fresh weight (µmol TE/g FW).

2.1.4.7. Phenolic Compound determination

The total phenolic content of the okra pod was determined by using the Folin-Ciocalteu assay [19]. Folin-Ciocalteau (FC) colorimetry was based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. Sodium carbonate solution (100 ml) was used in a volumetric flask. The spectrophotometer was set to 765 nm. A reagent blank was prepared. 1 ml of Folin–Ciacalteu's phenol reagent was also added to the mixture. The solution was diluted with distilled water and mixed then incubated at room temperature. The absorbance against reagent blank was determined with a UV-Vis Spectrophotometer Lambda 5 and expressed as mg gallic acid equivalent (GAE)/ 100g fresh weight.

2.1.4.8. Vitamin C determination

Vitamin C (Ascorbic acid) concentration was determined by applying a redox titration having potassium iodate in the presence of potassium iodide. 1 ml of titrant was utilized for each flask and calculated.

2.1.4.9. Minerals content measurement

Analysis of mineral contents of okra (K^+, Ca, Mg, Na, and Fe,) was done using an Atomic Emission (AE) spectroscopic multi-element analyzer (MOA). Samples were grounded properly using a green pod by mortar and pestle. The 5ml water was mixed with the sample. After that 1ml of the sample was injected into the MOA and readings were calculated.

2.2. Experiment 2

Plant materials and seed cultural operation and measurement of residual effects of NAA in 2nd year (April-October): In this experiment, the same procedure was used as Experiment 1 mentioned above. The residual effects of NAA (25, 50, and 100 mg/l) on aborted seeds (seedless) mainly have been observed in the second year. Fruit set percent, pod size, weight, and some biochemical content like chlorophyll, vitamin C, and total antioxidants were investigated.

2.3. Statistical analysis

The obtained data were statistically analyzed using the SPSS Computer Program. The data were analyzed following the Analysis of Variance (ANOVA) technique and mean differences were adjusted by using Duncan's Multiple Range Test (DMRT) at a 5% level of significance.

3. Results

3.1. Experiment 1: pod production, yield contributing characters, and seed yield

Higher pod set was found in 25 and 50 mg/l concentrations than in 100, 200, and control (Fig. 2). Results in Table (1) have observed that pod length, pod diameter, pod size, and pod weight, were significantly influenced by different concentrations of NAA. The data revealed that 25 mg/l produced the longest pod (10.25cm) followed by 50 (5.21cm), 100 (2.72cm), 200 (0), and control (3.2 cm) respectively. Pod diameter was found maximum with 25 mg/l and 50 mg/l followed by 100 mg/l and water control. Significantly highest pod size was obtained at 25 mg/l followed by 50, 100 mg/l, and water control. In this respect, pod weight was recorded as significantly highest at 25 mg/l (16.2g). The second heaviest pods were obtained at 50 mg/l (5.31g) followed by 100 mg/l and water control. TSS and vitamin C were found highest in the 25mg/l concentration (Table 1).

Pod harvested from 100 mg/l treated plants had significantly highest aborted seeds percentage (56%) and it was

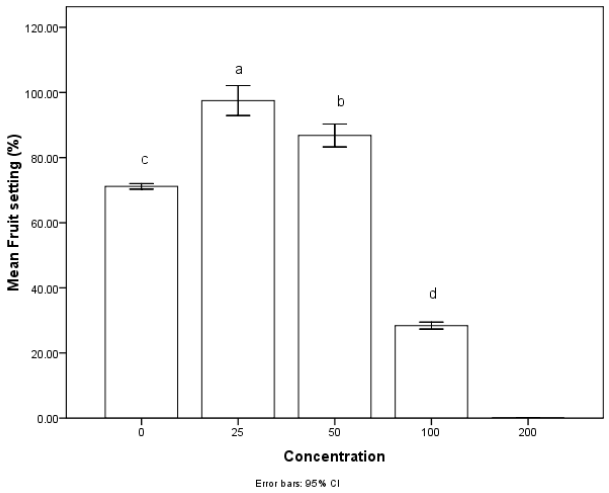


Fig. 2. Effect of NAA at different concentrations on fruit set of okra applied by ovary stigma injection in the first year. Means followed by same letter or no letter do not differ significantly at the 5% level by Duncan's Multiple Range Test (DMRT).

Table 1. Effect of NAA on physical characters and biochemical composition of okra pod in the first year.

Concentrations (mg/l)	Pod length (cm)	Pod diameter (cm)	Pod size (cm^2)	Pod weight (g)	Soluble solid (%Brix)	Ascorbic acid (mg/100g)
0	3.19±0.02c	1.66±0.02c	5.31±0.10c	1.92±0.03d	2.29±0.020b	10.74±0.01c
25	10.25±0.02a	2.22±0.024a	22.53±0.26a	16.20±0.02a	2.77±0.020a	15.78±0.02a
50	5.21±0.02b	1.72±0.019b	8.99±0.12b	5.31±0.02b	2.39±0.011b	11.32±0.02b
100	2.72±0.03d	1.23±0.03d	3.37±0.11d	2.76±0.02c	2.13±0.02c	10.40±0.03d
200	0	0	0	0	0	0
LSD (0.05)	0.03	0.03	0.21	0.02	0.02	0.029
	*	*	*	*	*	*

Values are means ± standard deviation. Means followed by same letter or no letter do not differ significantly at the 5% level by Duncan's Multiple Range Test (DMRT).

followed by 50 mg/l control and 25 mg/l. Control and 25 mg/l treatments had increased the production of healthy seeds (Table 2). 25 mg/l treatment which had maximum healthy seeds per pod followed by water control and 50 mg/l while no healthy seeds were observed in 200 mg/l treatment. Results in Table (2) indicated that chlorophyll content was affected significantly by different concentrations of NAA. The results showed that all concentrations of NAA (25, 50, and 100 mg/l) increased chlorophyll content. The seed yield of okra as influenced by NAA at different concentrations in the first year was found significantly differences in both aborted and healthy seeds (Fig. 3).

Total moisture, fibers, flavonoids, carotenoids, total phenol, and antioxidants (DPPH) were found to be higher in the 25 and 50mg/l concentrations than in other concentrations (Table 2). The highest mineral content was found at 25 mg/l concentration (Table. 3). The second highest was observed at 50 mg/l concentration. Fig. 4 Shows the seeded and seedless okra pod at the concentration of NAA 100mg/l in the first year. Fig. 5 shows the residual effects of Control (seeded) and seedless okra pod at the concentration of NAA 100mg/l in the 2nd year.

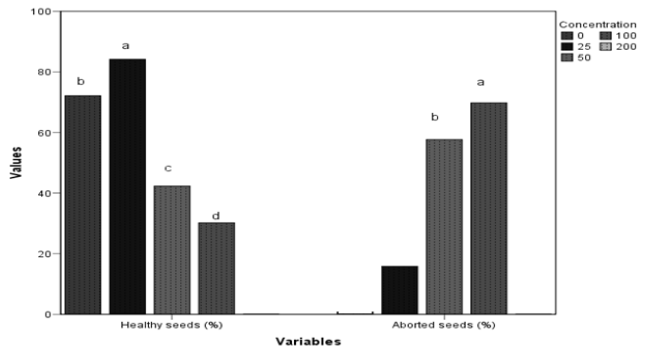
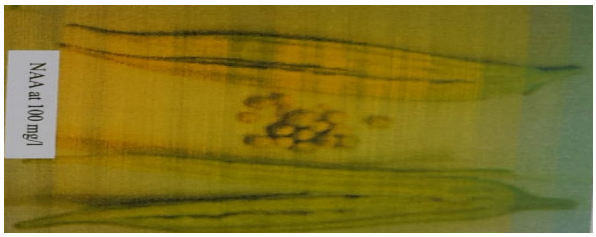


Fig. 3. Seed yield of okra as influenced by NAA at different concentrations in the first year. Means followed by same letter or no letter do not differ significantly at the 5% level by Duncan's Multiple Range Test (DMRT).

A. Control (Full seeded okra pod)



B. Seedless okra pod as affected by NAA 100mg/l

Fig. 4. Visual comparison of seeded (A) and seedless (B) okra pods resulting from NAA application (100 mg/l) via ovary stigma injection in the first year.

Table 2. Fiber, moisture chlorophyll, and bioactive compounds determination in the first year.

Concentration	Fiber (%)	Moisture (%)	Chlorophyll	Flavonoid (mg/100g)	Carotenoid (mg/gFW)	Antioxidant (DPPH) (µmole TE/gFW)	Phenol Content (mg GAE/100g)
0	25.0c	53.0b	40.0±0.4d	48.5c	9.30c	15.40c	98.0b
25	30.2a	65.0a	60.1±0.4a	105.7a	18.98a	22.70a	116.0a
50	27.1b	63.0a	52.9±0.7b	79.3b	11.80b	18.30b	115.0a
100	26.9b	55.0b	49.1±0.4c	78.4b	10.70b	17.70b	103.0b
200	00.0	00.0	28.5±0.6e	00.0	00.0	00.0	00.0

GAE: Assumed to be Gallic Acid Equivalents. **TE:** Assumed to be Trolox Equivalents. **FW:** Assumed to be Fresh Weight. **± Notation:** Represents standard deviation or error. **Letters (a, b, c, d, e):** Indicate statistical significance or groupings.
Values are means ± standard deviation Means followed by same letter or no letter do not differ significantly at the 5% level by Duncan's Multiple Range Test (DMRT).

Table 3. Effect of different concentrations of NAA on mean mineral element contents of okra applied by ovary stigma injection in the first year

	Minerals				
Concentration	**K+**	**Ca**	**Mg**	**Fe**	**Na**
0	91.53±0.03c	56.02 ±0.02a	39.18±0.02a	0.409±0.001b	5.15±0.03b
25	93.93±0.05a	56.15±0.02a	39.51±0.02a	0.445±0.002a	6.31±0.01a
50	92.30±0.02b	55.92±0.02b	39.20±0.02a	0.424±0.001a	6.0±0.01a
100	91.49±0.02c	55.40±0.02b	37.45±0.01b	0.408±0.002b	2.61±0.03c
200	0	0	0	0	0
LSD (0.05)	0.04	0.22	0.03	0.02	0.03
	*	*	*	*	*

Values are means ± standard deviation Means followed by same letter or no letter do not differ significantly at 5% level by Duncan's Multiple Range Test (DMRT).

Table 4. Residual effects of NAA at different concentrations on Pod set, size, weight, and biochemical content in the second year (April-October, 2020). Means followed by the same or no letter do not differ significantly at the 5% level by Duncan's Multiple Range Test (DMRT).

Concentrations (mg/l)	Seedless (%)	Pod size (cm²)	Pod weight (g)	Chorophyll (SPAD)	Total antioxidant (DPPH)TE/ gFW	Ascorbic acid/ Vitamin C (mg/100g)
Control seed (0)	0	5.0±0.10c	1.8±0.03d	40.1±0.4d	15.3±0.030c	10.6±0.01c
Aborted seed at 25	10.5±0.02a	22.0±0.2a	16.1±0.03a	59.1±0.3a	2.4±0.030a	15.6±0.03a
Aborted seed at 50	41.6±0.03b	8.1±0.1b	5.0±0.01b	52.6±0.6b	17.5±0.01b	11.1±0.02b
Aborted seed at 100	55.2±0.02d	3.5±0.2d	3.0±0.02c	48.7 ±0.4c	17.1±0.02b	10.6±0.03c

B. NAA 100 mg/ml A. Control (seeded)

Fig. 5. Residual effect of NAA on seedless okra pods in the second year: control (A) vs. 100 mg/L treatment (B).

In the second year, the residual effects of aborted seed (seedless) (at different concentrations) when hormone-treated seed was grown and found there was a decreasing trend of most of the parameters like pod weight, size, seedless percent, leaf chlorophyll, antioxidant (DPPH) and Vitamin C (Table 4). However, in comparison to the control, NAA concentrations showed better residual effects in the second year (Fig. 5).

4. Discussion

This study compared the bioavailability of different concentrations of NAA for improving growth, yield, and fruit quality when applied to okra crops by flower ovary stigma injection technique. Plant growth regulators (PGRs) are becoming an increasingly important aspect of agricultural and horticulture practices for many cultivated plants [20]. Several reports indicate that application of the plant growth regulators can provide germination, growth, fruit set, fresh vegetable weight, and seed yield quality [1, 21]. It was reported [21] that both natural and synthetic plant growth regulators control plant activities and production through the regulation of specific physiological processes. However, Gibberellic acid is safe for human health and can be used for different aims [22]. Plant growth regulators play a central role in the morphology and physiology of plants. The effect of the growth regulator depends on plant species, variety, growth stage, concentration of chemicals that are used, application technique, and frequency of application [23]. Application of NAA at 25 and 50 mg/l increased the pod growth over control applied by flower ovary stigma injection method. Plant growth regulators were concerned with enhancing cell division and elongation [24]. The hormone treatment of IBA-HMSNPS (Indole butyric acid-Hollow Mesoporous Silica Nanoparticles) at a concentration of 3 mg/l exhibited the highest values for root length, root fresh weight (1.03 g), and dry weight (0.07 g), respectively [25]. It was stated [26] that increased stem or fruit elongation might be due to the stimulating action of GA3, which alleviated the cell wall by increasing its plasticity [27]. The results confirm those [28] and [29] who found that growth regulator applications increased the plant growth of soybean and red sorrel, respectively. However, both investigations found that GA_3 at 100 ppm was more efficient than IAA. Also, studies reported that GA_3 increased plant height in various crops; soybean [30] and some cowpea cultivars [28]. It was reported [29] that GA_3 application increased branch number by breaking apical dominance [31]. It was stated that GA_3 delayed the loss of chlorophyll [32]. In addition, it is reported that increasing yield might be related to plant height, leaf number, and leaf area. They also stated that branches/plant is an important factor that offers a chance for the plant to grow more flowers and higher pods. GA_3 allows water to enter the cells of fruits and dissolved materials which leads naturally to an increase in fruit size by increasing the permeability of the fruit cell wall [33, 34]. IAA and GA_3 application at 100 ppm increased the yield of cereal crops [35, 36] respectively. Therefore, NAA is also the same group as IAA and the mode of action is the same as IAA and GA_3.

5. Conclusions

From the results, it can be summarized that 25 and 100 mg/l of NAA concentrations were the best for okra pod growth, and biochemical, and bioactive compound development. The 25 mg/l concentration was the best for pod growth and development as well and 100 mg/l was the best for seedless (aborted) okra production in the first and second years. Also, the residual effects of aborted seeds were found to have a decreasing trend of most of the parameters like pod weight, size, seedless percent, leaf chlorophyll, antioxidant (DPPH), and Vitamin C in the second year. However, NAA concentrations showed better residual effects in the second year in comparison to the control. Therefore, it can be recommended that the okra flower ovary stigma injection technique be used commercially in the vegetable industry. The internal application of flower ovary stigma injection can reduce chemical and production costs without hazardous environmental pollution.

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