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Effects of different plant growth regulators on the pod setting, aborted seed, nutrient, and vitamin C content in okra using flower ovary injection

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ABSTRACT

The study was conducted to investigate the effect of different concentration of indole acetic acid (IAA), naphthalene acetic acid (NAA) and gibberellic acid (GA_3) on okra pod setting and size, soluble solids content (SSC), vitamin C and mineral contents using flower ovary injection method. The fruit set significantly increased in IAA and GA_3 compared to the NAA at 100mg/L concentration. NAA had lower fruit setting than the IAA and GA_3 treated plant. The higher pod (fruit) size was obtained in GA_3 (18.8 cm^2) as compared to the IAA (8.98) and NAA (8.72 cm^2) at 100 (mg/L) of concentration. Moreover, the highest SSC was obtained by 100 mg/L of IAA concentration as compared to the GA_3 and NAA. The maximum vitamin C was found in IAA and GA_3 as compared to 100 mg/L NAA concentration. On the other hand, the higher mineral contents like K, Ca, Mg, Na and Fe was found in 100 mg/L IAA and GA3 concentrations than 100 mg/L NAA concentration. The higher concentrations of IAA and NAA greatly decreased the healthy seed percentage as compared to the lower concentration. However, GA_3 increased the healthy seed percentage at 50 and 100 mg/L then decreased at 200 mg/L. Aborted seed percent was increased from 25-100mg/L IAA and NAA and then decreased at 200 mg/L. however it was found increasing trend from 25-200mg/L GA_3. It seemed that GA_3 and IAA were better growth regulator as compared to the NAA for okra pod production and development of nutrient content.

Keywords: Fruit size, vitamin C, minerals, SSC, okra, growth regulators.

INTRODUCTION: Okra is one of the superlative and nutritious vegetable in tropical and subtropical area in Asia and Africa (Hossain *et al.*, 2021). It has a great nutritional and medicinal value in the field of vegetable industry. It belongs to the family Malvaceae, genus, *Abelmoschus and* species, *esculentus.* It was reported that total production of okra was 5.9 m tons in the world (Vekaria *et al.*, 2017). The okra pods are rich source of vitamin A, B and C complex and calcium (Ca), potassium (K), phosphorus (P). Okra pod comprised of many nutritional contents which are essential for human health. About 100 g of fresh pod comprised of moisture (89.6%), K (103 mg), Ca (90 mg), Mg (43 mg), P (56 mg) and vitamin C (18 mg) (Lee *et al.*, 1990).

Recently, the application of plant growth regulartors are known as one of the most important treatments in agriculture. Some horticulture crops productions were increased by application of different growth regulartors (Jafarullahet *et al.*, 2007). Growth regulators mainly regulate the plant physiological and biochemical processes and play a vital role in dormancy, organ size, flowering, fruit set and regulation of chemical composition of plants (Noor *et al.*, 2017). The phytohormone auxin affects 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 (Leyser, 2006). There are more than 100 distinct gibberellins produced primarily in roots & young leaves but GA_3 or gibberellic acid is the most popular available form. It has many effects on plant growth such as enhance stem and internodes elongation, produce seed germination, enzyme production during germination and fruit setting and growth (Davies, 1995) and breaking of dormancy. Taniguchi *et al.* (2018) indicated that plant growth regulators might be used to regulate the vegetative growth of plants. Application of IAA increased the plant height, number of internodes, leaf area, dry weight of shoot and dry weight of Gram plant respectively (Sarkar *et al.*, 2002). However, work has been done on the use of GA3 to improve vegetative growth, pod size, and delay pod maturity in vegetables using spray method. But no studies have been conducted to evaluate the complete profile of vegetative growth, pod yield in response to GA_3, IAA and NAA application to okra using ovary injection.

OBJECTIVES: The effect of different concentration of GA_3, IAA and NAA at different concentrations on okra pod setting and size, vitamin C, TSS and minerals, healthy and aborted seed were investigated. Also, to evaluate the efficacy of this injection method (treated ovary) of application on the okra pod.

MATERIALS AND METHODS: The present study was conducted at the commercial farm in Banting, Selangor, Malaysia and the soil in this field was peat with pH 6.6. Okra (F_1) were sown in the experimental area of Banting. These seeds soaked in sterile distilled water for 24 h. and spread on sterile moist filter paper in sterile Petri-dishes. The Petri-dishes were incubated in the dark cupboard at 28ºC. Okra seeds (3 seeds/hill) were sown directly into the soil by hand in the soil fertilized with NPK 19 g/plant. Thirty days after emergence, sidedress with 10g/hill and plots were irrigated. The experiment was laid out in randomized block desig (RBD) with four replications. The entire field was divided into 15 blocks and each block into 20-unit plots. The size of the unit plot was 1 x 1 m^2. The seeds were shown in the rows with the help of hand plough. The gaps where seeds failed to germinate were filled up within 2 weeks after germination of seeds. After field preparation, seeds were sown in well-prepared seedbeds in line with a distance of 70 cm when germination completed thinning was done to maintain the plant-to-plant distance of 30 cm. The depth of planting was 1cm from the surface of the soil. Hoeing, weeding and other cultural practices were conducted uniformly.

Preparation of plant growth regulators: The growth regulartors (IAA, GA_3 and NAA) were employed in the experiment with 0, 25, 50, 100 and 200 mg/L. The GA_3, IAA and NAA were dissolved separately in 2mL of ethanol (1%) and distilled water was added up to 100 mL. Sterile distilled water was used as a control.

Application of treatment: About 1.5mL of GA_3, IAA and NAA (25, 50, 100 and 200 mg/L) were applied to the flower ovary with the help of sterile injecting and 4 flowers were selected randomly from each replication. From each treatment, 5 pods were selected randomly to measure the size (cm^2), length (cm) and diameter (cm). Healthy and aborted seed were measured by visual observation of seed production. Four flowers were selected randomly per each replication (figure 1).

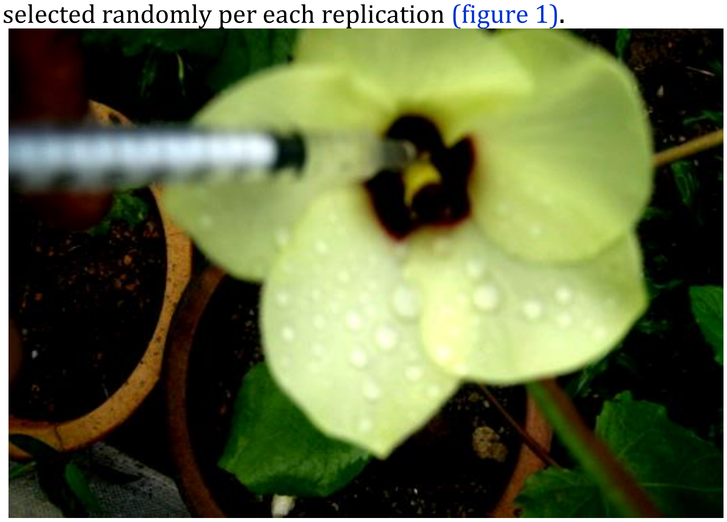

Figure 1: The ovary injection technique after anthesis in okra plant

Concentration of Vitamin C: It was computed with a redox titration having potassium iodate in the presence of potassium iodide. Ascorbic acid was oxidized, and excess iodine was free to react with starch indicator, following the blue-black starch-iodine complex. About, 1 mL of titrant was utilized for each flask and calculated. The measurements were taken that obtained and averaged them.

Average volume = Total volume / number of trails X mL of iodine solution / 0.250 g Vit C = X mL iodine solution / X mL Vit C = g Vit C in that sample.

Total soluble solids content (TSSC): It was evaluated at 25ºC with the help of refractometer. The TSS were expressed with Brix (Atago ATC-1, 32-10 Honcho, Itabashi-ku, Tokyo 173- 001, Japan). A few drops of juice were kept on the refractometer prism surface and reading was collected from skin pad.

Potassium content: After harvesting the pods, K content was measured with Cardy Potassium meter instantly. The pods (1 g) was homogenized in sterile distilled water (5 mL), centrifuged at 4000 rpm for 10 min. 3 drops of supernatant were shifted onto the calibrated sensor pad (Cardy Potassium Meter, Model-2400, USA), having a sampling paper placed on the sensor. The reading in was recorded.

Minerals content: Analysis of mineral contents (Ca, Mg, Na, and Fe,) was conduted using Multi element analyzer (MOA). Samples were grounded in sterile water (5mL) and 1mL was injected to the MOA to mearuse the mineral contents.

Statistical analysis: The obtained data were statistically analysed using SPSS Computer Programme, Version 16. The data were analyzed following Analysis of Variance (ANOVA) technique and mean differences were adjusted by using Duncan's Multiple Test (DMRT) at 5% level of significance.

RESULTS: A significant variation was recorded in the pod set and size due to the application of GA_3 and IAA at 100mg/L of concentration (table 1). Fruit/pod set was influenced by the application of GA_3, NAA and IAA (table 1). The IAA and GA_3 influenced the pod/fruit set significantly ($P<0.05$). The GA_3 greatly increased the fruit set as compared to the IAA and NAA at 100mg/L of concentration. The analysis of variance showed that GA_3 exhibited highly varied influence on fruit size. Results in table 1, indicated that pod size, were significantly affected by the GA3. The higher pod/fruit size was obtained in GA3 (18.83 cm^2) as compared to the IAA and NAA (9.0 & 8.7 cm^2) at 100 (mg/L) of concentration. Pod size was found significantly highest with 100 mg/L of GA3 concentration.

Ascorbic acid (vitamin C) content and seed percent: One of the most important parameters for pod quality is the vitamin C of pod for human consumption. The vitamin C in okra pods were significantly increased by IAA ovary injection treatment (table 1). Vitamin C pod content was increased at 100 mg/L, IAA as compared to the GA_3 and NAA. The IAA at 100 mg/L resulted in the highest pod content of vitamin C while ovary injection treatment had the least effect on vitamin C with GA_3 and NAA. The concentrations of GA_3 (50 and 100 mg/L) greatly increased the healthy seed percentage as compared to the 25 and 200 mg/L concentration (figure 2).

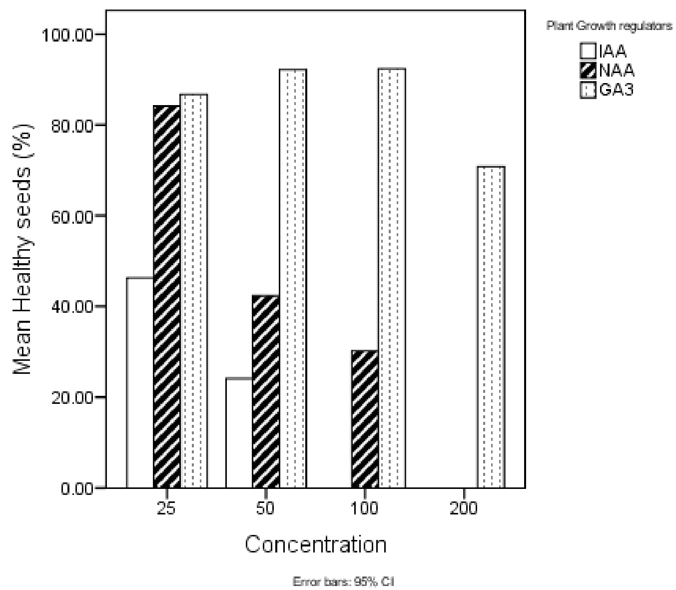

Figure 2: Change of healthy seed yield production of okra *A. esculentus* as a function of various concentrations of IAA, NAA and GA3 with ovary injection treatment.

However, IAA and NAA decreased the healthy seed percentage at higher concentration (100 & 200mg/L). The higher aborted seed (seedless) in IAA and NAA than GA3 at 50 and 100mg/L concentrations (figure 3).

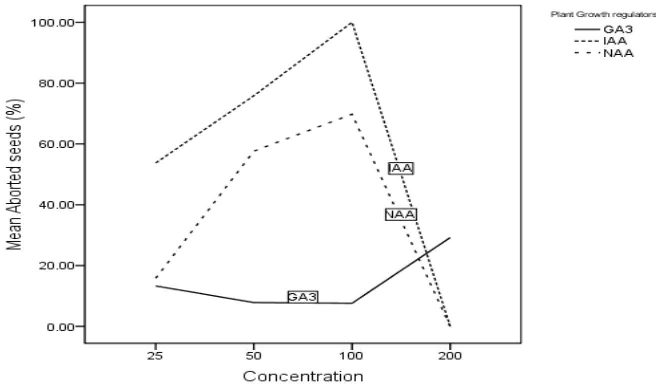

Figure 3: Effect of IAA, NAA and GA3 at various concentrations on the production of aborted seeds(Stenospermocarpy) in okra plant under ovary injection treatment.

The highest seedless percent was observed at 100mg/l IAA as compared to the NAA and GA_3. Seedless (aborted seed) percent was found increasing trend from the concentration of 25, 50 and 100mg/L and then decreased at 200mg/L in the case of IAA and NAA. However, it was found decreasing trend from the concentration of 25, 50 and 100mg/L and then increased at 200mg/L concentration in the case of GA_3 (figure 3).

Total soluble solids and nutrient: One of the most important parameters for pod quality is the soluble solids content of pod. Total soluble solids (TSS) were markedly increased by GA_3 and IAA application (table 1). Under GA_3 treatment okra pod presented the highest TSS was with 100 mg/L at the same time, the lowest value was in NAA treatment. There are some important minerals highly concerned with human nutrition, such as K, Ca, Mg, Fe and Na were analyzed in the samples of okra pods. Flower injection application of okra plants with IAA and GA_3 at 100 mg/L led to obvious increase in the endogenous content of K (table 1). The highest value of K was obtained from the application of 100 mg/L GA_3 (93.3mg/100g) followed by IAA and NAA (93.2mg/100g), and (92.8mg/100g) respectively. Calcium content in pods ranged from 59.12 to 59.4 mg/100g (table 2).

With respect to the effect of IAA application, IAA at 100 mg/L had a positive effect on calcium pod content and showed the highest Ca (59.4mg/100g). Also, Mg, and Na content significantly were increased by IAA application. On the contrary, Fe content increased significantly by the application of GA_3 with 100 mg/L compared to IAA and NAA (table 2).

DISCUSSION: Utilization of the plant growth regulators has been an increasingly magnificent factor in the practices for many cultivated plants (Hossain *et al.*, 2021). Several reports which observed that application of the plant growth regulators influenced growth, fruit set, fresh vegetables weight and pod yields quality (Gemede *et al.*, 2015). Application of IAA and GA_3 at 100 mg/L increased the fruit setting and size over NAA applied by ovary injection method. It was reported that GA_3 was considered as enhancing the cell division and elongation

Concentration	Na	Fe	Mg	Ca
IAA	6.2±0.02a	0.43±0.02c	39.3±0.03a	59.4±0.2a
GA3	6.12±0.01a	0.462±0.01a	39.3±0.0b	59.2±0.01ab
NAA	5.94±0.01b	0.454±0.02b	38.55±0.02c	59.12±0.01b
LSD 0.05	0.021	0.037	0.016	0.02

Table 1: Effect of IAA, NAA and GA3 ovary injection (100 mg/l concentration) on fruit setting percent, fruit size and ascorbic acid of okra.

Growth regulators	Fruit setting (%)	Fruit size (cm^2)	Ascorbic acid (mg/100)	K+ (mg/100g)	Soluble solid (% Brix)
IAA	49.29±39.25b	8.98±8.18b	13.78±1.94a	93.2±0.01a	2.81±0.02a
NAA	49.10±38.66b	8.72±8.87b	9.38±6.06b	92.8±0.02b	2.76±0.02b
GA3	79.44±20.86a	18.83±5.42a	9.6±5.97b	93.3±0.03a	2.82±0.03a
F(Between groups)	4.21	9.10	3.86	4.11	9.11

Table 2: Nutritional elements (mineral contents) of okra pods as affected by IAA, NAA and GA3 at 100 (mg/l) applied by pre-sowing treatment.

(Harrington *et al.*, 1957). Veer Kumar (2002) and Sreedhar (2003) stated that increased stem elongation might be due to the stimulating action of GA_3, which alleviated the cell wall by increasing its plasticity. The results confirm with those of (Sarkar *et al.*, 2002) and Mukhtar (2008) who observed that GA_3 and IAA application increased the plant growth and yield of soybean and red sorrel, respectively. But both investigations found IAA at 100 ppm was more efficient than GA_3 and NAA. Also, earlier studies reported that growth regulators increased plant and fruit growth in soybean, sesame, and cowpea (Hilli *et al.*, 2010). With GA_3 at 100 mg/L concentrations, there was a significant differences in pod in comparison with IAA and NAA. GA_3 increased fruit number per plant in Bell pepper (Mukhtar, 2008) found that GA_3 and IAA treatment at 100 ppm increased leaves number and leaves area and chlorophyll content in *Hibiscus sabdariffa L.* Also, Thakur *et al.* (2018) mentioned a significant increase in the leaf length in onion by application of GA_3. This might be attributed that GA_3 and IAA increased the division and elongation of the cells led to better vegetative growth of plants. In addition(Wanyama *et al.*, 2006) reported that GA_3 application increased branches number by breaking apical dominance. Jordi *et al.* (1995) informed that GA_3 delay the loss of chlorophyll. Moreover, IAA and GA_3 at 50 and 100 mg/l had better effect than lower concentrations (25 and 50 mg/l) and control. GA_3 and IAA developed yield and physiochemical characters of leafy vegetable (Deore and Bharud, 1990). In addition, Deore and Bharud (1990) reported that increasing yield might be related to the plant height, leaf number, leaf area. Another reason might the physiological role of gibberellin and indole acetic acid in increasing cell division and elongation and stimulating the complete growth of plant which revealed in better pod setting by using of IAA and GA_3. IAA and GA_3 allow water to enter the cells of fruits and dissolved materials which lead naturally to increase fruit size by increasing the permeability of fruit cell wall (Abduljabbar *et al.*, 2007). IAA and GA_3 application at 100 ppm increased the yield of rice and soybean as well as seedless of okra (Hossain *et al.*, 2021) respectively.

CONCLUSION: From the above discussion it can be concluded that 100 mg/l of GA3 was showed better effect for pod setting and size than IAA and NAA. IAA was exhibited better than GA3 and NAA for vitamin C development. For aborted seed (seedless) production, 100 mg/l IAA was the best concentration compared to the GA3 and NAA all concentrations. So it can be recommended that ovary injection technique can be used commercially in the vegetable industry. The internal application as ovary injection can reduce the chemical and production cost without hazardous any environmental pollution.

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