CHAPTER 1

INTRODUCTION

Okra is a tall growing, annual, semi woody and warm season crop (Balock, 1994). It is self- pollinated, but occasionally up to 20% cross pollination happens by insects (Grubben, 1977). The okra flowers blossoms only one day. Okra pods are harvested when they reach the maximum size but still tender (may be 60-180 days from sowing) around 5-10 days after opening of flower depending on the cultivar grown (Adetuyi *et al*., 2008).

Okra pods are considered nutritious, providing some human supplementary vitamins such as vitamin C, A, B- complex, calcium, potassium, iron and other minerals (Lee *et al*., 1990; Adebooye and Opunta, 1996). Okra pod contains many nutritional contents which important for human health. One hundred gram of fresh pod has around; moisture (89.6 percent), 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 (Markose and Peter, 1990).

The application of plant growth regulators is known as one of the most important treatments used nowadays in agriculture. Some horticulture crop production were increased by application of different growth regulators (Jafarullahet *et al*., 2007). Growth regulators mainly regulate the plant physiological and biochemical processes. For example, play a major role in dormancy, organ size, crop improvement, flowering and fruit set, regulation of chemical composition of plants and control of mineral uptake from the soil (Nickell, 1978). Some groups of them are naturally occurring, organic substances that affect the plant growth when used at low concentrations, and sometimes they act as inhibitors at high concentrations

(Jules *et al*., 1981). There are some reports, which indicate that application of growth regulators improved the growth and yield of vegetables (Mukhtar, 2008; Hernandez, 1997; Ashraf *et al*., 1987, 1989).

Plant growth regulators could manage vegetative and reproductive growth balance. PGRs are known as chemical messengers because they are produced in one part of plant and affect on another part. Exogenous of plant growth regulators improved the yield production and fruit quality of horticulture crops (Emongor, 1997). At the cell level, hormones join to a protein receptor that sends a signal down a transduction pathway to switch on exacting genes. Throughout transcription and translation this guides to production of an enzyme protein which actually reasons the change in plant growth (Arteca, 1996; Wolfe, 1993). There are five major classes of plant hormones and each one of them has multiple effects on plant growth and development. The five classes are: Auxin, Gibberellic acid, Cytokinins (CKs), Abscisic acid (ABA), and Ethylene.

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 (Leiser, O., 2006). In addition, auxin is synthesized in mature leaves but very little amount. Auxins play an important role in cell elongation (Schneider, 1938), encourage cell division with CK, induce differentiation of xylem and phloem in vascular tissues (Jacobs, 1952), flower initiation, sex determination, fruit development, parthenocarpic fruits and promote cell wall loosening at very low concentrations. Furthermore, auxin is used in-plant cell culture at concentration 0.1 to 10 mg/l to regulate callus initiation, growth and induction of embryogenesis (Vanderhoff and

Dute, 1981). Plant growth regulators have many beneficial effects on plant growth. One of them is increased the activity of antioxidant enzymes such as ascorbate peroxidase, glutathione reductase, catalase and peroxidase, which defends plant from chilling (Dwyer *et al*., 1995), high temperature, photo inhibition (Sopher *et al*., 1999; Fletcher *et al*., 2000), SO2, salinity (Sadhu and Gupta, 1997), ozone injury (Fletcher *et al*., 2000). Moreover, PGRs induce changes in the contents of cytokinin, ethylene and polyamines (Fletcher *et al*., 2000). One of the most important auxin produced by plant is indole-3- acetic acid (IAA). IAA is produced in cells in the bud and young leaves of a plant. Plant cells mainly produce IAA from tryptophan but can also produce it independently of tryptophan. Also, there are some artificial auxins often affect plant growth in the same way that IAA application. They are used commercially more than IAA because they are cheaper and more stable such as naphthalene acetic acid (NAA) is used to control fruit set and growth of fruit and vegetable plants. The second group of plant growth regulators is Gibberellins which is natural plant hormone. Gibberellins were named for a genus of fungi that produce the same chemical and cause "foolish seedling" disease (Yabuta, 1935). 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. GA_3 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; Karssen *et al*., 1989) and breaking of dormancy. Latimer, (1991) indicated that plant growth regulators may be used to regulate the vegetative growth of plants. The most commonly used PGRs are IAA, NAA and GA_3. Leopold, (1962); Tukey, (1954); Weintraub and Norman, (1949) reported that some synthetic and naturally plant growth regulators enhance various types of plant growth. Hayashi, (1940); Wittwer and Bukovak, (1957) found that application of auxins compounds increased the rate of seed

germination, root initiation, stem elongation, leaf growth, flowering fruit set, maturation, and ripening. Also, they found that gibberellins treatment enhanced the various types of plant growth. Application of GA_3 increased the plant height, number of internodes, leaf area, dry weight of shoot and dry weight of Gram plant respectively (Khan and Rashid, 1983). Also, application of NAA at different concentrations enhanced the vegetative growth and grain yield of cowpea (Resmi and Gopalakrishnan, 2004).

Okra pods have the large number of seeds that many people find distasteful, as well, Improvement of seedless fruit can be attained by chemically stimulated parthenocarpy. There are many ways available to generate parthenocarpic fruits in many crops. Exogenous application of natural and synthetic auxins to unpollinated flowers caused the formation of parthenocarpic fruit in some horticulture crops such as cucumber, tomato, bottle gourd, brinjal, *Cucurbita*, watermelon, etc. (Homan, 1964; Elassar *et al*., 1974; Takashima and Hatta, 1955; Miyazaki, 1965; Terada and Masuda, 1941; Schwabe and Mills, 1981). In addition, gibberellins and cytokinins applications induced seedless fruits in some horticulture crops (Choudhury and Phatak, 1959; Elassar *et al*., 1974; Kulkarni and Rameshwar, 1978; Yu, 1999; Hayata *et al*., 2000). In addition, exogenous application of auxins to unpollinated flowers of tomato improved fruit growth and in other horticulture crops (Nitsch, 1952).

A lot of work has been done on the use of PGRs to improve vegetative growth, fruit size, and delay fruit maturity in vegetables. However, no studies have been conducted to evaluate the complete profile of vegetative growth, fruit quality and seeds yield in response to growth regulator application to okra. Keeping in view the importance of fruit quality in okra and the role of plant PGR's innovative methods of application in improving vegetative growth, fruit

size, fruit quality, nutrient contents and seeds yield, this study was carried out to evaluate the growth and chemical responses of okra to some growth regulator treatments and to determine the optimum concentrations of the hormones that can be recommended for applied to vegetable for enhanced growth and quality. It was also carried out to evaluate the optimal method of exogenous application of plant hormones this has the best effect on vegetative growth, fruit quality. The overall objective of the experiments was to improve the productivity and quality of okra (*Abelmoschus esculentus*) which will benefit our local farms. There is a little if any information on plant growth regulators (PGRs) application on okra in Malaysia and Saudi Arabia, and was no literature found on the methods employed, especially with okra.

RESEARCH OBJECTIVES

The research objectives of the study were:

1. To investigate the effect of different concentrations of IAA, NAA and GA_3 on the external morphology i.e. stem, leaves, chlorophyll content, maximum quantum yield (Fv/Fm) , flowering, fruiting and seeds yield axes along with their effect on chemical composition of the pods (Vitamin C, K, Na, Fe and Ca) .
2. To establish the optimal concentrations of these hormones that can be recommended for applying on this vegetable for enhancing plant growth, yield, quality of pods and their nutritional contents.
3. To evaluate the efficacy of different application methods of the plant growth regulators for promoting plant growth, pod yield and pod quality in okra.
4. To examine the effect of exogenous auxins and gibberellins with for inducing parthenocarpy or Stenospermocarpy (aborted seeds) in okra pods.

CHAPTER 2

LITERATURE REVIEW

2.1 GENERAL DESCRIPTION

Okra (*Abelmoschus esculentus* L. Moench) is considered as one of the most important vegetable crops grown in tropical and subtropical regions of the world (Tindall, 1986). Okra could thrive on well in nearly all tropical regions and the relatively warmer temperate regions (Plate 2.1). Furthermore, okra is one of the common and very much tasty vegetables throughout the tropical and subtropical areas in Asia and Africa (Bisht and Bhat, 2006). On the other hand, many countries cultivate and grow okra as commercially crop like India, Turkey, Iran, Western Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Burma, Japan, Malaysia, Brazil, Ghana, Ethiopian, Cyprus and the southern United States (FAOSTAT 2008). In different parts of the world, okra is known as lady's finger in England, gumbo in the United States of America, guino-gombo in Spanish, guibeiro in Portuguese, bhindi in India, bendi in Malaysia and bamia, bamya or bamieh in Middle East (Ghauhan, 1972).

Okra is easy to cultivate as a garden crop as well as on a large commercial farm (Plate 2.2). It is cultivated for its immature pods, which can be used as a fried or boiled vegetable in soup and stews, also used as dry pods, canned products, or frozen for all year round. In some countries like West Africa, the immature leaves, buds and flowers can be consumed. Sometimes the leaves are used as Spanish or cattle feed. The dried seeds of okra pods are roasted, ground and consume as a coffee in some countries. In fact, the dry seeds contain 18-20% oil and 20-25% crud protein (El-shaikh and Mohammed, 2009; Reddy *et al*., 1997). Furthermore, the seed oil can be used in soap and beauty industry while the protein can be

used for fortified feed preparation. The fiber of okra is used in jute; textile and paper manufacture (Sankaran and Singh, 2006). The roots and stems are used for cleaning cane juice while preparing gur (Chauhan, 1972).

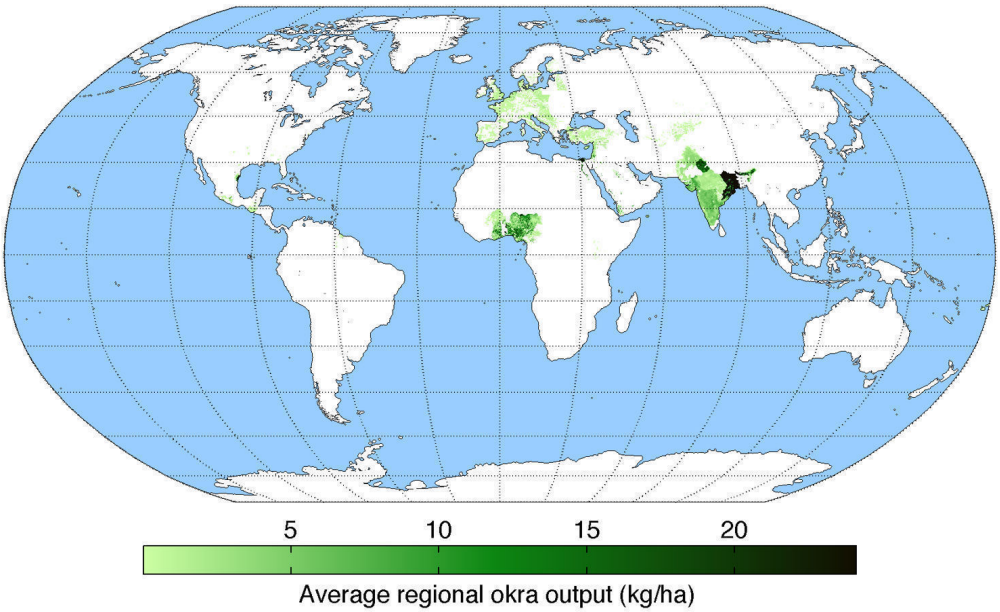

Plate 2.1. Worldwide okra production.

Plate 2.2. Photograph shows okra (*Abelmoschus esculentus* L.) plants.

2.1.1 Taxonomy

Taxonomic classification is shown in table 2.1. In the earlier; cultivated okra and related wild species belonging to genus Hibiscus, section *Abelmoschus* in the family Malvaceae (Linnaeus, 1753). Since its calyx, corolla and staminal column are fused together and fall down at anthesis (caducous); it was renamed as *Abelmoschus esculentus* L. (Kundu and Biswas, 1973; Terrell *et al*., 1974; Bates, 1968; Van Borssum and Van, 1966). The *esculentus* name means edible in the Latin language and *Abelmoschus* means father of musk, which derived from Arabic language having musk-scented seeds.

Table 2.1. The classification of Okra plant (NBPGR, 1990)

Name	**Okra**
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Malvales
Family	Malvaceae
Genus	*Abelmoschus*
Species	*esculentus*

2.1.2 Origin and Distribution

Okra is discovered by a Spanish Botanist in Egypt in 12th century. It is one of the oldest cultured crops (Shujat *et al*., 2006). The Egyptians were cultivated okra in the Nile valley and were used it as food. Kochhar (1986) reported that okra was found growing wild about the Nile River in Egypt as well as Ethiopia. However, *Abelmoschus* genus clearly shows overlapping in West Africa, South Asia and Southeast Asia, which considered as the center of diversity (Van Borssum 1966; Siemonsma, 1982; Bisht, *et al*., 1995; Duzyaman, 1997). It was propagated then through North Africa to the Mediterranean, the Balkans, and India. It was cultivated then in Americas at Brazil in 1658 before extending in the United States in 1781 (Saenz, 1960). Some authors argued that okra originated in tropical Africa (Purseglove, 1974) and others said that it was originated in tropical Asia (Grubben, 1977). In Africa, slaves used ground okra as a part of their diet, which led to use ground okra seeds as a coffee by southerner people during American Civil War blockades of the 1860s. Okra is becoming

increasingly common and well-known in Western Countries. *Abelmoschus esculentus* can be shown all around the world from Mediterranean to equatorial areas (Plate 2.3).

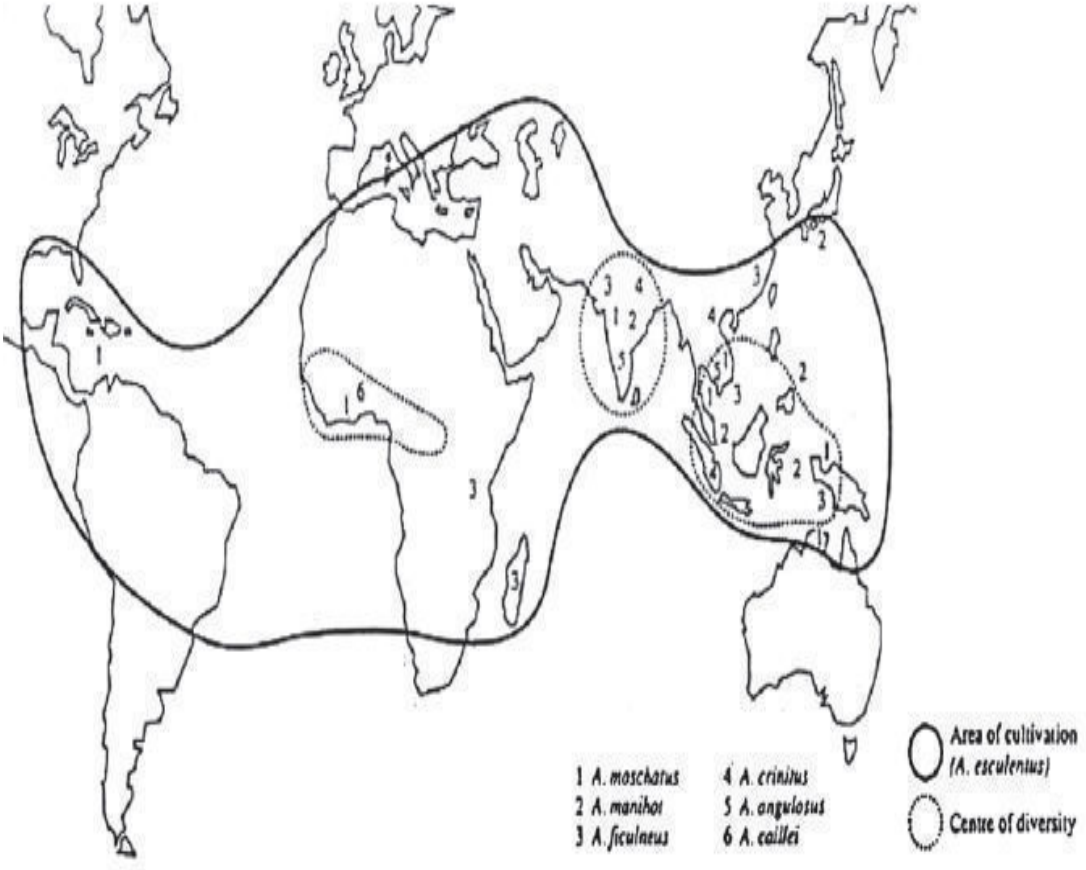

Plate 2.3. Geographical distribution of *A. esculentus* species modified from Charrier (1984).

2.1.3 Botany

Lady's finger is propagated by seeds with duration 90-100 days. It is an annual herbaceous plant and well adapted to a wide range of soil types (Balock, 1994). The chromosome number of *Abelmoschus esculentus* has been reported as 2n=130 (Skovsted *et al*., 1953). This species has a deep tap root (Ugwoke and Onyishi, 2009) while its stem is semi woody, erect, stout and sometimes pigmented with a green or radish tinges color (Plate 2.4). Its stem reaches heights from 0.91 m in dwarf pods to 2 m in other varieties (Tripathi *et al*., 2011). The leaves of okra are lobed and are generally hairy, some reaching up to 30 cm in length, and they are heart-shaped and simple (Plate 2.5). Leaves are dark green and be similar to maple leaves (Grubben and Denton, 2004). Okra's attractive blossoms are yellow, funnel-shaped and be like hibiscus flowers (Plate 2.6). Okra's flower is large around 5 cm in diameter, with five yellow petals with a purple spot at the base of each petal, and flower will last only for a day. Pollen fertility is higher in the period between one hour before and after opening of a flower (Purewal and Randhaw, 1947; Purseglove, 1968). Although okra flowers are perfect (male and female reproductive parts in the same flower) and self-pollinating, the showy blooms are very attractive to bees, and varieties readily cross-pollinate up to 20% (Gurbben, 1977).

When stored under ideal conditions, okra seed could remain viable for about five years. Each flower expands small green pods. The fruits or pods are capsule (5-35 cm long and 1-5 cm in diameter) and their color normally is green. The surface of pods is covered by soft hair. These pods are the edible portion, are harvested when they reach maximum size but still tender (Plate 2.7). Okra pods include numerous seeds, gray to black (Tripathi *et al*, 2011). These seeds have a chunky coat and do not germinate easily (Plates 2.8 and 2.9).

Plate 2.4. *Abelmoschus esculentus* stem with purple tinges.

Plate 2.5. *Abelmoschus esculentus* leaves.

Plate 2.6. Okra flower

Plate 2.7. Photo shows okra (*Abelmoschus esculentus*) immature pods

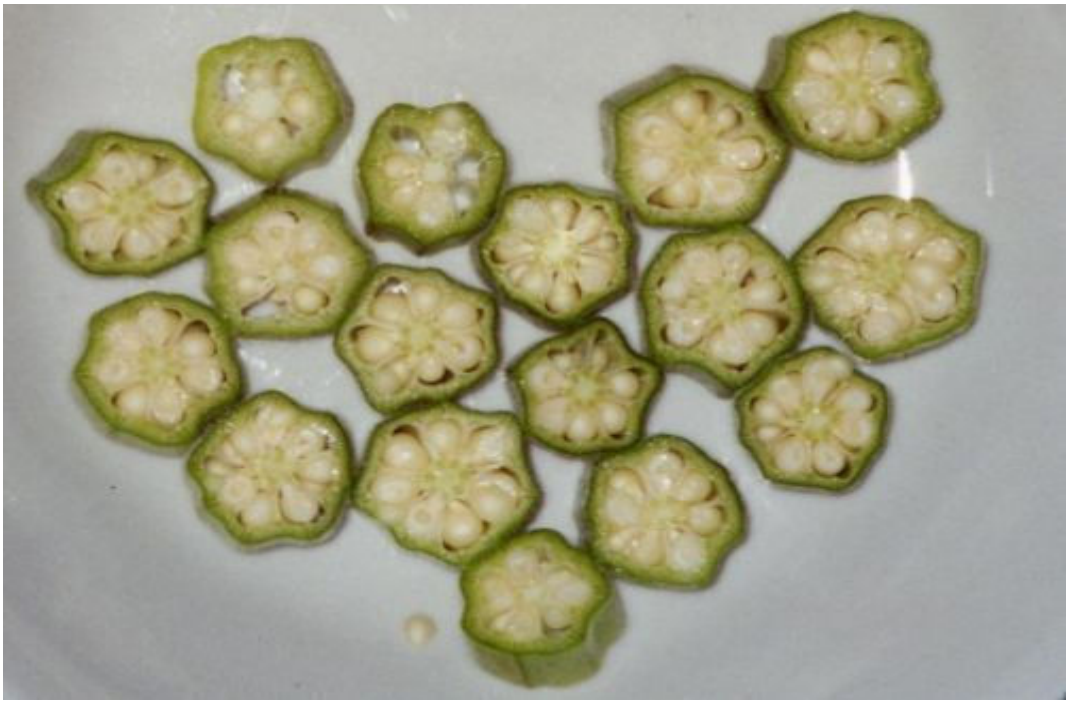

Plate 2.8. Photograph shows okra seeds

Plate 2.9. Photo shows pod seeds

2.1.4 Pod nutrition

Okra plays an important role in the human diet where its edible fresh pods a good source of vitamins, especially A, B6,C (Table 2) and has high value of some nutritional elements such as iron, calcium (FAO, 1988).

Table 2.2. Okra nutritional value per 100 grams serving of fresh raw pods (USDA National Nutrient database).

Nutrient	Amount
Energy	1.5%
Carbohydrates	7.03 g
Protein	2 g
Dietary Fiber	9%
Total Fat	0.1
Cholesterol	0 mg
Vitamin C	21.1 mg
Sodium	8 mg
Potassium	303 mg
Iron	0.80 mg
Selenium	0.7 mcg
Zinc	0.60 mg
Magnesium	57 mg
Manganese	0.990 mg
Calcium	81 mg
Copper	0.094 mg
Phosphorus	63 mg

2.1.5 Health benefits

In addition, okra has many health benefits for human body. Adebooye and Opunta, (1996) reported that both gum and pectin which okra fresh pods supplied them; they had medicinal potentials for human like; the establishment of healthy blood vessels and the prevention of high blood pressure. Furthermore, it was reported that okra's mucilage provided some

medicinal applications such as an emollient, laxative and expectorant (Muresan and Popescu, 1993). In addition, okras bind surplus cholesterol and toxins (in bile acid) carrying dumped into it by the filtering liver (Siemonsma and Kouame, 2004; Kochhar, 1986; Shalau, 2002), Okra is also a good source of fibers as signified in the treatment and avoidance of colon cancer. Moreover, Nadkarni (1927) mentioned that bhindi is a very useful for genitor-urinary disorders and chronic dysentery. Furthermore, it was used for healing ulcers and relief from hemorrhoids (Adams, 1975). According to Bangana and Dossou (2005), the water-soluble fiber of some fruits and vegetables has been the focus of scientific research in relation to possible health benefits to cardiovascular diseases. The three-weeks randomized crossover placebo study was carried out between 30 healthy subjects concluded that okra was an effective cholesterol lowering nutritional adjunct. Also, okra has many benefits for our health such as:

I. The superior fiber found in okra helps to stabilize blood sugar as it curbs the rate at which sugar is absorbed from the intestinal tract.

II. Many alternative health practitioners believe all disease begins in the colon. The okra fiber, absorbing water and ensuring bulk in stools, helps prevent and improve constipation. Fiber in general is helpful for this but okra is one of the best, along with ground flax seed and psyllium. Unlike harsh wheat bran, which can irritate or injure the intestinal tract, okra's mucilage soothes, and okra facilitates elimination more comfortably by its slippery characteristic many people abhor. In other words, the incredibly valuable okra not only binds excess cholesterol and toxins (in bile acids) which cause numerous health problems if it is not evacuated, but also it is assured easy passage out of the body of same.

III. Okra fiber is a good source for feeding the good bacteria (probiotics). This contributes to the health of the intestinal tract.

IV. It helps to retain most of okra's nutrients and self-digesting enzymes

V. It is one of the important vegetable for those feeling weak, exhausted, and suffering from depression.

VI. It is used for curative ulcers and to keep joints limber. It helps to neutralize acids, being very alkaline, and provides a temporary protective coating for the digestive tract.

VII. It treats lung inflammation, sore throat, and irritable bowel syndrome.

VIII. Okra has been used successfully in experimental blood plasma replacements.

IX. It is good for summer heat treatment.

X. It is helpful to reduce constipation.

XI. It is better in normalizing the blood sugar and cholesterol level.

XII. It is effective for asthma. Okra's vitamin C is an antioxidant and anti-inflammatory, which curtail the development of asthma symptoms.

XIII. It is better for atherosclerosis.

XIV. It is believed to protect some forms of cancer expansion, especially colorectal cancer.

XV. Eating okra helps to support the structure of capillaries.

XVI. Some information shows that eating okra lowers the risk of cataracts.

XVII. It is better for preventing diabetes.

XVIII. Okra defends the human bodies from pimples and upholds smooth and beautiful skin" (Zook, 2006).

2.2 THE USE OF PLANT GROWTH REGULATORS

As previously mentioned, okra (*Abelmoschus esculentus*) is one of the important economic vegetables where it has nutritional content and medicinal potentials. In addition, the increase in demand of fresh vegetables, there is an urgent need of boosting up the production, quality and nutrients content of this crop. The use of the plant growth regulators

(PGRs) is becoming an increasingly important aspect in agricultural and horticulture practices for many cultivated plants (Monselise, 1979). Because there are many reports, which indicate that application of the plant growth regulators can provide germination, growth, fruit set, fresh vegetables and seed yields quality (Saimbhi, 1993). Plant growth regulators (PGRs) are one of the most useful tools for horticultural crops to enhance yield, improve crop quality and management (Emongor, 1997).

Growth regulators have a positive effect on fruit quality, carbohydrate, protein, vitamin contents and mineral elements content. Conversely, the plant hormones or phytohormones is produced naturally by plant tissues whereas plant growth regulators are created by human or extracted from plant tissues (Dascaliuc and Ralea, 2002). In particular, phytohormones are a natural compounds cause significant variation in growth and improvement when apply to the plants at very low concentrations (Soliman, 2006), whereas Siddiqui and Krishnamoorthy, (1991) observed that higher concentrations of the growth regulators decreased the dry matter and yield, which could be inhibited the metabolic pathways. However, the actual reason following this could be clarified if quantitative and qualitative analysis of some important enzymes occupied in different metabolic pathways could have been monitored sometimes during the diverse growth phases of the crop (Fattah and Wort, 1970).

Additionally, Moore (1984) and Salisbury and Ross (1992) informed that production of plant growth regulators occurred at one part then they transported to a specific target tissue where they initiated response. These compounds were applied to the plants via many techniques like foliar spray; seed soaked; flower spray before anthesis or after anthesis, stem injection, flower injection and useful in-plant tissue culture medium. Both plant hormones

and plant growth regulators are organic compounds (Grubben, 2004). Crane (1964) and Nitsch (1970) reported that phytohormones regulated the growth, ripening of fruits. In addition, they played an important role in plant's physiological and biochemical procedures such as dormancy, organ size, flowering and fruit set and regulation of chemical composition of plants (Nickell, 1978). Resmi *et al*., (2004) reported that plant growth regulators (PGRs) application increase fruit set and flowering in vegetable crops. In addition, Hernandez, (1997); Ashraf *et al*., (1987, 1989) found that application of plant growth regulators improved plant growth and crop reproduction. Provosoli and Carlucci (1974) reported that external application of plant growth regulators affected the growth and development processes of several algae. Furthermore, external application of phytohormones was reported to affect the morphogenetic and physiological growth in higher plants (Steward, 1972; Krishnamoorthy, 1981). Some of these effects were in roots, stem, and leaf, initiation of flowering, sex expression, fruiting, dormancy, parthenocarpy and senescence. The application of plant growth regulators improved fruit quality, length, diameter, yield, ripening as well as fruit color (Jawanda and Vij, 1973; Sunjavi and Phadnis, 1973; Lal and Thakur, 1979; Malasi, 1981).

The plant growth regulators have certain changes in metabolism through fruit and seed growth as a result of which there would be better accumulation of food reserves resulting in higher seed yield. These valuable effects of chemicals were also informed by Das and Das (1995) in pumpkin, Sitaram *et al*., (1988) and Rafeekher *et al*., (2002) in cucumber, Gedam *et al*., (1998) in bitter gourd and Balaraj (1999) in chilli. The aim of growers and researchers similar is able to influence the vegetative and reproductive growth of crop plants. Now-a-

days, plant growth regulators are possibly the greatest tools available for reaching this goal. Successful application of PGRs can resolve production problems.

Plant growth regulators have been used effectively as foliar sprays to enhance flowering, harmonize bloom, or change the time of flowering to evade unfavorable climatic environments or to change harvest to a time when the market is more economically favorable. Foliar spry of PGRs are regularly used to develop fruit set, decrease June drop or to avoid pre-harvest drop to increase yield. Plant growth regulator sprays are applied to enhance fruit size directly by motivating cell division or to enhance fruit size not directly by reducing a fruit number through the application of PGRs that decrease the number of flowers formed or encourage flower or fruit abscission. PGRs have been applied as both pre- and post-harvest treatments to accelerate or slow the ripening development, color improvement, and maturation of specific fruit tissues to develop the quality of the product sold in the market. More recently, achievement has been achieved using PGRs to even out alternate bearing and increase increasing yield for multiple alternate bearing cycles. The emerging use of PGRs to overcome the undesirable effects of biotic stresses is increasingly successful (Cavusoglu and Kabar, 2007).

Plant growth regulators play a major role as a tool for improving the agriculture and consumption of tomatoes due to increasing the nutritional value and the potentials of this crop and thus serve as advancement towards the achievement of global food security (Olaiya *et al*., 2010). The influence of plant growth regulators depend on the plant species, variety, their growth stage, and concentration of chemicals, application method and frequency of application (Hilli *et al*., 2009). Mohammad Golam *et al*., (2008) found a positive relative

between the fruit size and weight with PGRs concentrations. In addition, seedless induction was encouraged by applying exogenous auxins in many crops such as cucumber, tomato, bottle ground, watermelon, ect (Homan, 1964; Elassar *et al*., 1974; Miyazaki, 1965; Terada *et al*., 1941). However, Plants can store extreme quantities of exogenously applied hormones in the form of reversible conjugates, which release the active hormones when and where plants need them during the growth period (Davies, 1987).

Many years ago some biologists studied five major groups of plant hormones are generally recognized namely, auxins, gibberellins, cytokinins, abscissic acid, and ethylene (Table 2.3). These hormones adjust the growth and the development of fruits at various stages (Ozga and Reinecke. 2003). Amzallag *et al*., (1990) found that plant growth regulators played an essential role in the combination of the response expressed by plant under environmental stress. Plant hormones managed the plant growth tuberization and yield in potato (Bodlaender and Algra, 1966; Racca and Tizia, 1968; Dimalla and Van Staden, 1977; Kumar *et al*., 1974, 1977). Kumar *et al*., (1981) found that chemical composition of potato was changed by the application of plant growth substances. Likewise, Vamil and Agnihotri, (2010) observed that application of indole acetic acid, indole-3-butyric acid, 2, 4-dichlorophenoxyacetic acid; naphthalene acetic acid, gibberellic acid and cytokinin at rates 10µM and 100µM on *Bambusa arundinaceae* increased the germination percentage, seedling vigor, leaf area and chlorophyll content while these applications reduced the germination time. The highest leaf area and chlorophyll content were observed by IAA 100 mg/l and10 mg/l concentrations, respectively.

In general, auxins, gibberellins and cytokines encourage growth, and they are known as growth promoters while abscissic acid has a negative effect and cause inhibition, and it is known as growth inhibitors. Ethylene is a gas which causes fruit ripening, and leaf abscission (Moore, 1998). However, auxin and gibberellins encourage the cell elongation and enlargement; the cytokinens which motivate cell division; ethylene gas which stimulates the swelling growth of stem and roots, and the inhibitors.

Table 2.3. Major plant hormones and plant growth regulators (Gregory, 1998).

Plant Hormones	Plant Growth Regulators
Auxins Indoleacetic acid (IAA) Phenylacetic acid (PAA) 4-chloro-indoleacetic Acid (4-Chl IAA) Indoleacetonitrile (IAN) Indoleproprionic acid (IPA) Indolebutyric acid (IBA) Gibberellins Gibberellic acids, 1 > 100 Cytokinins Zeatin, Isopentenyl adenine Zeatin riboside Abscissic Acid Ethylene Others Fusicoccin, Brassins, Turgorins Salicyclic Acid, Jasmonic Acid Batasins	Auxins Naphthalene acetic acid (NAA) 2,4-D 2,methyl-4-chlorophenoxyacetic acid (MCPA) 2,4,5-T Cytokinins Kinetin, Benzyladenine

2.3 AUXINS (IAA AND NAA):

Auxins are a class of hormone or growth substance found in plants. They were the first plant hormones discovered, and they are most important for controlling growth and morphogenesis in plant tissue and organ culture. The term auxin is derived from Greek word "auxein" which means "increase or to grow" (George, 2008). Charles Darwin and his son Francis were considered to be the scientists responsible for the beginning of current research in plant growth regulators on phototropism described in a book which called "The Power of Movement in Plants. They found that light fell on the tip of a grass coleoptile, from one side caused the effect to be transmitted down due to which the coleoptile curves to the light. When the coleoptile tip was detached phototropic response did not occur (Darwin, 1880). This compound was the first remote from plants by a graduate student in Holland named Fritz Went (Went, 1928). This growth regulator was named by Went. Kogl and Haagen-Smit (1934) isolated the first plant hormone and was later named indole-3-acetic acid (IAA).

2.3.1. Influence of natural and synthetic auxins on various species of plant

Auxins are a common class of the plant hormones which can cause cell elongation tissue swelling, cell division, formation of adventitious roots, callus initiation and growth, induction of embryogenesis and encourage cell wall loosening at very little concentrations (Vanderhoff and Dute, 1981). Auxins are produced in the meristems at shoot tips or root tips and expanding tissues of a plant such as the growing buds and embryos (Johri, 2001). The movement of auxins is a polar, which means that auxins created at the shoot tips where the meristems located can only be transported downwards and towards the bottom of the plant, but not upwards. However, auxins produced at the root tips is only transported upwards and

towards the main body of the plant. In the higher plant, auxins transported long distances extensively through the Phloem.

The cell wall of plant cells is made of cellulose, which has a structure that made the cell wall very unbending, and so even there is an intake of water into the cell; it will not elongate. Auxins, after being transported to the target tissue, motivate the cells there to elongate by providing an acid medium. The acid medium can encourage the rigid cell wall to enlarge easily since the acid can weaken the inflexible structure of the cellulose, and when the cell intakes water, the cell can lengthen (Byrne, 1999). The procedures of auxins that cause cell elongation and play an essential role on plant development (Zahir *et al*., 2007). The auxins are known for their influence to enhance cell growth (Westwood, 1993; Arteca, 1996; Davis, 2004). In addition, male sterility can be induced in some crops by using auxin and gibberellin treatments such as sunflower (Frank and Koves, 1977). Auxins encouraged female flowering, and gibberellins increased male flowering in monoecious cucurbit plants (Galun, 1962; Peterson and Anhder, 1960; Rudich and Halevy, 1974). Similar results have been found in *Cannabis sativa* (Mohan *et al*., 1970), *Carica papaya* (Ghosh *et al*., 1975; Jindal and Singh, 1976) and other plants (Bhandari and Sen 1973; Corley, 1976). There are naturally-occurring auxins include 4-chloroindole-3-acetic acid (4-Cl-IAA), phenylacetic acid (PAA) and indole-3-butyric acid (IBA) (Plate 2.10). Furthermore, there are synthetic auxin analogs include 1-naphthaleneacetic acid (NAA), 2, 4-dichlorophenoxyacetic acid (2, 4-D), and others (Plate 2.11). One familiar type of auxins is indole-3-acetic acid (IAA).

Kende and Zeevaart, (1997) reported that Indole-3-acetic acid (IAA) as the primary auxin in higher plants, encouraged basic processes, for instance, cell elongation and division. It was formed by the conversion of an amino acid by enzymes. All the actions were caused by

auxins mainly contributed to the naturally-occurring auxin IAA (George, 2008). The most important auxin produced by plant is indole-3-acetic acid (IAA) with wide physiological effects (Lambrecht, 2000). To function as a regulator of cell expansion, IAA concentration in the target tissue must be controlled. Because the response of plant cell to the auxin depends on the auxin concentration and location. For example, if a specific concentration of auxin encourages shoot growth in one plant, the same concentration will usually inhibit the shoot growth in its root growth (Pilet, 1985).

IAA present in plant such as indole-3-pyruvic acid, tryptamine (Cooney and Nonhebel, 1991) or tryptophan (Rayle and Purves, 1967; Percival *et al*., 1973). Indole-3-acetic acid (IAA) is mostly the most important plant auxin. It is a heterocyclic compound that is a phytohormone called auxin. This colourless solid the molecule is derived from indole, containing a carboxymethyl group (acetic acid). In addition, IAA is mostly synthesized at young leaves and buds in cell of the plant. Mostly, IAA is synthesized from tryptophan in the plant cells (Herbert *et al*., 1973). The molecule is derived from indole, containing a carboxymethyl group (acetic acid). Gillaspy *et al*., (1993) informed that fruit growth was controlled by varies phytohormones such as IAA and cytokinins.

IAA has many different effects on the cell enlargement by promote cell enlargement and stem growth. Moreover, auxin encourages a cell division in the cambium, in mixture with cytokinins, in tissue culture. Auxin motivates differentiation of phloem and xylem. As well auxin may inhibit or promote leaf and fruit abscission. Moreover, auxin has a significant effect on plant growth, seed germination, fruit quality, yield production. Many studies presented the effect of IAA on the agriculture crops. For example, Schneidar *et al*., (1967)

found that IAA manipulated the initiation of the reproductive phase in bryophytes. IAA had been shown to increase the flowering, fruit set, total dry matter of crops (Gurdev and Saxena, 1991). Application of IAA also increased the plant height, number of leaves per plant, fruit size in groundnut (Lee, 1990) cotton (kapgate *et al*., 1989), cowpea (Khalil and Mandurah, 1989) and rice (Kaur and Singh, 1987). A spray of IAA at 25 and 50 mg/l concentrations increased number of leaves; shoot dry weight, number of produced flowers per plant, number and weight of pods and seed per plant in cowpea. At the same time, 50 and 100 mg/l considerably decreased the number of flowers abscised from cowpea plant (El-saeid *et al*., 2010). Elbassiouny and Shukry (2001) found that foliar spray of IAA increased the plant height, fresh and dry weight, number of branches and number of leaves per plant in addition to yield components (pods per plant, seeds per pod, weight of pod, weight of seeds per plant and weight of seeds/Feddan). It has been shown, in many plants, that auxin application increased yields (Mandurah, 1984; Rao *et al*., 1997). Furthermore, IAA application increased plant height, stem girth, leaf development and chlorophyll content obtained in *Hibiscus sabdariffa* L (Mukhtar, 2008). In addition, IAA application to the *Abelmoschus esculentus* and Solanum gilo increased vitamin A, B6 and C contents.

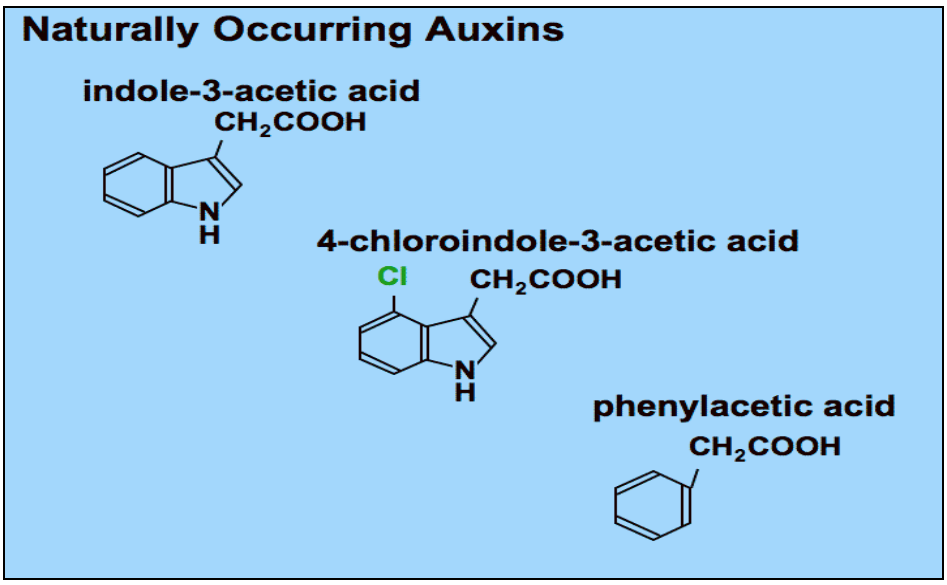

Plate 2.10. Gallery of native auxins: 4-chloroindole-3-acetic acid (4-Cl-IAA), Phenylaceticacid (PAA) and indole-3-butyric acid (IBA)

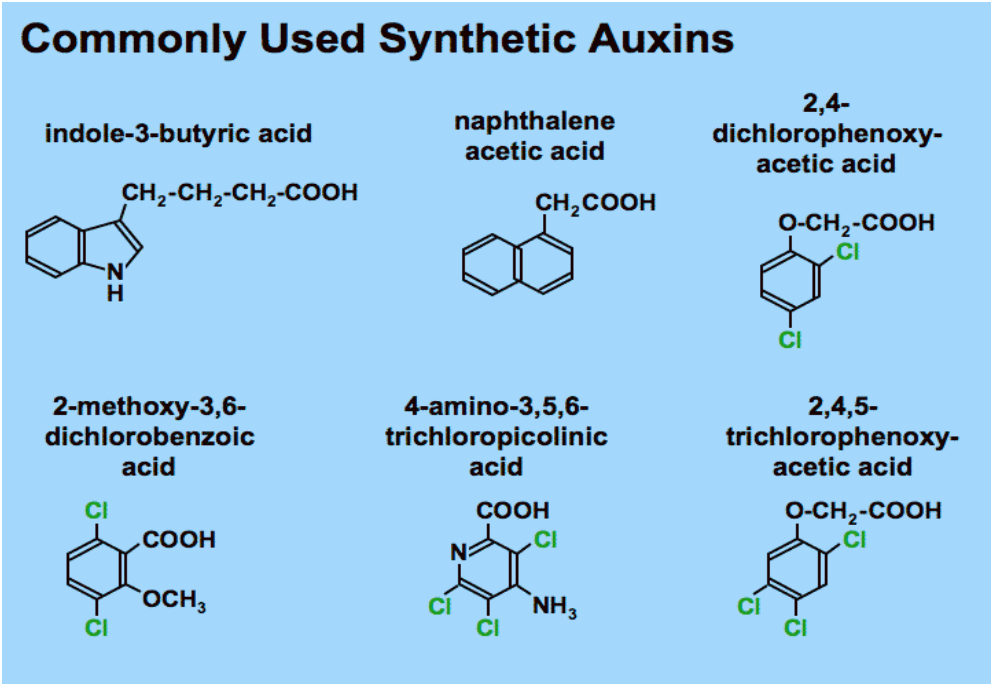

Plate 2.11. Synthetic auxins

Several studies have been reported that flower induction was done resulting from a combination of IAA and salicylic acid in different Lemnaceae (Cleland and Ajami, 1974; Watanabe and Takimoto, 1979; Khurana and Maheshwari, 1980; Kaihara *et al*., 1981; Fujioka *et al*., 1985). Flowering response to the treatment effect of GA_3 and IAA was caused

by 20 mg/l nicotinic acid in *Lemna paucicostata* and *Lemna gibba* (Lemnaceae). Furthermore, Jaiwal and Bhambie, (1983) reported that application of IAA increased the leaf blade in *Cicer arietinum*. IAA could also encourage or reduce or inhibit floral initiation in some plants. Reduction has been reported for a wide variety of species, such as barley, soybean, xanthium, and kalanchoe (Lang, 1952; Liverman, 1955). Even so, IAA induced flowering in pin apple (Clark and Kerns, 1942). Foliar application of GA_3 and IAA increased total number of flowers, fresh pod yield, fresh fruits per plant and per cent fruit in okra plant (Rattan *et al*., 1987). The increase in the number of flowers was as a result of the acceleration of axillary buds into new shoots providing extra sites for more flowers and the growth regulators also play a major role in preserving the flowers by its abscission retarding effect (Jacobs, 1955). Sarkar *et al*., (2002) worked on soybean and sprayed IAA three times with two different rates. They found that application of IAA at concentration 100 mg/l increased the high plant height, number of flowers, number of pods, percentage of fruit set, number of seed per plant, seed yield per plant and seed yield (t /ha) at the same time as IAA at 200 gm/l increased number of branches, number of leaves, leaf area per plant and 100-seeds weight.

Application of IAA induced higher number of branches/plant (Chhipa and Lal, 1988). Moreover, Abdul *et al*., (1988) found that IAA increased number of leaves per plant in bell pepper. Both IAA and GA enhanced the number of pods per plant in groundnut, rice and gram (Lee, 1990; Awan and Alizai, 1989, Mange, 1971).

In pineapple plant, application of IAA at concentration 100 mg/l increased the percentage of fruit set (Going, 1956). In addition, 100 mg/l of IAA increased the seed yield in rice, sesame and soybean, respectively (Awan and Alizai, 1989; Sontakey *et al*., 1991 and Reena *et al*., 1999). Application of IAA 50 mg/l increased the shoot and root length, number of

branches, nodes and leaves in *Verbascum thapsus* (a medicinal Plant), whereas IAA 200 mg/l was the best treatment for increasing leaf area, number of flower and fruits. Furthermore, Bhandari *et al*., (2009), found that IAA at the rate of 50 mg/l was the best treatment for productivity of aboveground and underground compartment of *V. Thapsus* on annual basis. Several workers had been widely studied the role of growth regulators for improving, branching and enhancing different growth processes in many ornamental crops. Growth augmented and high-yield production was as a result of foliar spray of IAA (Sinha and Pal, 1983; Patil *et al*., 1987; Fuloria *et al*., 1990; Arora *et al*., 2000). Bose *et al*., (1980) found that IAA application increased shoot length in *Hippeastrum hybridium* and IAA treatment increased number of leaves per plant and leaf area (length and width). Parekh (1968) reported that IAA improved the growth of root in tomato. Similar results have been found in *Dendrocalamus stictus* by Mishra and Mishra (1982). Damian and Ross, (2002) observed that indole-3-acetic acid (IAA) encouraged the biosynthesis of the active gibberellin (GA1) in shoots of pea seedlings.

Furthermore, IAA application increased the root number, root length and root weight, bulb diameter, bulb weight and bulb yield of onion (Abdul Hey and Abdul Karim, 2002). Moreover, found that 200 mg/l level of IAA was highly affected on bulb diameter and bulb weight with a double dose. Similar results observed with application of IAA at concentration 200 mg/l increased the number of leaves in onion (Mathur, 1971). In addition, IAA improved the dry weight in some species of plants while GA_3 decreased it (Kumar *et al*., 1981). Bareen *et al*., (1988) reported that IAA application enhanced elongation and increased number of roots in garlic. Also, Ebofin *et al*., (2003) found that IAA treatment improved shoot elongation in *Parkia biglobossa*, *Senna siamea*, *Albizia lebbeck* and *Prosopis africana*.

Moreover, yield of rice and soybean in that order increased while sprayed by IAA 100 gm/l (Reena *et al*., 1999).

In bottle gourd, vine length extension was prompted by application of IAA at concentrations 50 mg/l and 100 mg/l respectively. IAA treatment increased the fruit size at low concentration at the same time as decreased it at the highest concentration (Vwioko and Longe, 2009). High proportion of germination was found in the low concentration of all growth regulators when they used in this trial except IAA application. Olaiya, (2009) reported that IAA, IBA and NAA increased the levels of crude proteins, crude fat, crude fiber, ash and titratable acidity in tomato fruits. These applications also improved necessary tomato nutrients, which are important in a human diet. But they clearly decreased the total carbohydrate content and dry matter at concentrations 60 mg/l of IAA, IBA, NAA and 100 mg/l of NAA. The treatments of 100 mg/l of IAA and 140 mg/l of IAA and NAA respectively reduced the pH of tomato pulp. Plant growth regulators can be enhanced the rate of germination of seeds and plant growth regulators similar to IAA; IBA and NAA accelerate fruit setting, ripening and reduce fruit dropping (Grierson and Kader, 1986).

Roberts and Osborne (1981) found that IAA influenced the development of the French prune (*Prunus domestica*) fruits. Ibrahim, (2007) observed that application of benzyl adenine and IAA reduced the flower abscission percentage, and they produced the highest number of pod setting in faba bean. In addition, he found that all of these bioregulators increased the protein content and total carbohydrate percentage of the produced seeds. Auxin or mixture with gibberellic acid (GA) and kinetin increased the germination of the seeds in radish and onion seeds (Thomas, 1976). Moreover, GA and IAA on higher plants caused elongation in

the primary cells in the young tissues and growth centers (Marschner, 1986). In this respect, both regulators increased the stem height of faba plant height (Shalaby and Ahmad, 1994; Abdel-Fattah, 1997).

Other investigators reported that application of growth regulators increased the number of branches/plant in faba plant (Abdel-Fattah, 1997). Many investigators reported that number of branches/faba bean plant was increased due to (Shalaby and Abdel-Halim, 1995) using kinetin; (Etman *et al*., 1991) using CCC; (Shalaby and Ahmad, 1994) using IAA, (Rashad and Ahmad, 1996) using GA_3; (Bekheta, 2000) using uniconazole and (Mahgoub *et al*., 2006) using paclobutrazole. In this respect, different growth regulators increased the number of flowers per plant in faba bean (El-Behiedi *et al*., 1991); (Rashad and Ahmad, 1996) and (Bekheta, 2000). Accord with these results, number of pods per plant was increased due to application of different growth regulators (El-Behedi *et al*., 1991; CCC (El- Quesni *et al*., 1992); IAA (Shalaby and Ahmad, 1994); GA (Abdel-Fatah, 1997) and uniconazole (Bekheta, 2000). Whittenberger and Nutting, 1948 reported that Zika (1939) and Malcher and Zika (1943) found presowing treatment of potato seed with Indoleacetic acid (IAA) led to the increasing tuber productions with average size and high potato starch.

The plant growth regulators such as Auxins, gibberellin and cytokinins enhance the fruit development in the absence of fertilization in several crop species, for instance, tomato and eggplants (Gillaspy, 1993). In this respect, when IAA and NAA applied directly to the ovary, auxins accumulated in it, which caused fruit set and following growth in *Cucumis sativus* (Kim *et al*., 1992). Several investigators also reported parthenocarpy was induced in Arabidopsis and in several agriculture species (tomato, eggplant, watermelon, and citrus) by

application of plant growth regulators such as auxin and cytokinins (Gillaspy *et al*., 1993; Vivian-Smith and Koltunow, 1999; Rotino *et al*., 1997; Carmi *et al*., 2003; Mezzetti *et al*., 2004) which suggesting the pivotal role of parthenocarpic that occurred with increasing auxin levels or responded in ovaries and ovules. Nitsch, (1952); Schwabe and Mills (1981) found that auxin control all developmental processes in plants, as well as fruit growth.

In another hand, natural and synthetic auxins applied exogenously to unpollinated flowers stimulate fruit growth in tomato and in other horticultural plants, indicative of that these hormones can replace the signals provide by pollination and fertilization. In addition, application of IAA and IBA caused the formation of parthenocarpic fruit in rapid-cycling *Brassica Rapa* (Schadler, 1994). Abd-Alaal *et al.,* (1982) observed that 2, 4-D, 2, 4, 5-T, 2, 4, 5-TP, IAA and GA_3 at the concentrations of 25-100 mg/l caused the induction of parthenocarpic fruit in the date plum. The best treatment to get the maximum proportion of seedless fruit was a single application of 50 mg/l IAA + 10 mg/l 2, 4, 5-T. Several theories about the role of auxins in causing parthenocarpic fruit have been offered. One sight holds that pollen and pollen tubes have a growth stimulator, maybe auxin, which was responsible for fruit development, mostly by motivating growth of the ovary (Dean, 1978).

The second viewpoint was that developing seed had auxin and causes growth of the fruit (Bandurski and Nonhebel, 1984). Ayala-Silva *et al*., (2003) found that gibberellic acid and indole-3-acetic acid encouraged growth in hemp, jute and kenaf in cotton, particularly fiber production and elongation. Also, application of IAA improved the fiber elongation and lint yield, fine fiber yield, chlorophyll content, and stem diameter, flowering and number of bolls in cotton, but it reduced the fiber strength, and fineness. As well, application of IAA

increased the potassium values in tomato (Salama *et al*., 1981). The effect of auxin at a cellular level increased the plasticity of the cell wall and the stimulation of respiration. Application of IAA induced branching with lush green color of leaves and caused late flowering and increased the number of floral buds (Naeem, 2004). IAA has an effect on the plant growth by enlarging leaves cells and increases the photosynthetic activities in plants. It also activated the translocation of carbohydrates during their synthesis (Awan *et al.,* 1999; Ritenour *et al.,* 1996). Qadeer (1996) found that foliar spray of IAA increased healthy leaf number in wheat plants. Das Gupta *et al*., (1994) recorded that foliar application of plant growth regulators like IAA and GA helped the plant to restore retardation in water content in Mungbean plants subjected to water stress.

The presowing treatment of cowpea seeds with IAA, GA and IBA showed that IAA increased the stem height of cowpea plant, but it doesn't have any effect on plant branch number. Furthermore, all of these growth regulators demonstrated a good germination, seedling growth, flowering and yield (Ogbonna and Abraham, 1989). Moreover, Midan *et al*., (1986) observed that IAA application improved the seed germination in onion. The similar results had been observed by Chhipa and Lal, (1988) in wheat seed. IAA induced many changes in plant growth at low concentration.

As mentioned above, natural plant growth regulators or synthetic controls the plant activities and their productions by controlling one or more of specific physiological processes within a plant (Lemaux, 1999; Olaiya and Osonubi, 2009). Indole-3-acetic acid (IAA) is one of the common and important natural auxins for vegetative growth, fruit production, fruit quality, fruit nutrient content, modifying sex expression, induce parthenocarpic fruit and

decrease abscission in different plants. For example, indole-3-acetic acid, gibberellic acid or kinetin at different concentrations encouraged the growth vigor like root length, root fresh and dry weight, shoot length, shoot fresh and dry weights and leaf area production of cowpea through the growth periods (Al-Desuqey, *et al*., 2007). Moreover, Sinsiri *et al*., (2007) found that IAA at different concentrations had a positive effect on root length, number of both roots and root hairs of cowpea, and they reported the best concentration of IAA was 300 mg/l.

Furthermore, there were some synthetic auxins which they had a positive effect on plant growth and fruit quality such as 1-naphthaleneacetic acid (NAA), 2, 4-dichlorophenoxyacetic acid (2, 4-D), indole-3-butyric acid (IBA) and others (Faust, 1989; Westwood, 1993). These auxins enhanced the fruit growth in several fruit species by their ability to increase cell size (Arteca, 1996; Westwood, 1993 and Davis, 2004). Several studies stated that a synthetic auxin was a useful in increasing fruit size. The most common synthetic auxins were naphthalene acetic acid (NAA) and indolebutyric acid (IBA), which were generally used in the nursery industry, and 2,4-dichlorophenoxyacetic acid (2,4-D). However, Naphthalene acetic acid (Plate 2.12) and indolebutyric acids are known to have a great influence on plant production processes (Salisbury and Ross 1992).

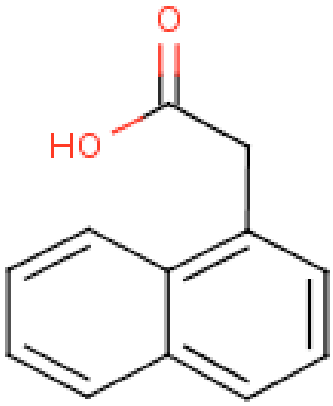

Plate 2.12. The Molecular Structure of 1-Naphthaleneacetic acid ($C_{10}H_7CH_2CO_2H$)

Younis and Tigani, (1977); observed that naphthalene acetic acid (NAA). It was one of the synthetic plant growth regulators who had been a significant effect on the fruit retention of many vegetables in addition horticulture crops and increased the yield significantly. The spray application of NAA at various concentrations has been reported for having a remarkable influence on fruit yield per plant, weight of tomato/fruit and number of fruit/plant of tomato and leaves nutrient contents. Tomato yield increased in this trail because of IAA application decrease the flower dropping (Alam and Khan, 2002). It also increased the yield/plant of chickpea (Karim, 2005). Several investigators as well reported an increase in yield of different plants such as tomato and cotton (Alam and Naqvi, 1989; and Younis and Tigani, 1977), by application of NAA at the time of flowering which preserves pre-harvest flower from dropping by increasing the endogenous auxin rate at this significant stage of reproductive development in tomato plant (Chandramony and George, 1976; Hays, 1957; Rao *et al*., 1977). Jafar Ullah *et al*., (2007) worked on cowpea using different rates of NAA and found that plant height, dry matter and yield were highly affected by NAA treatments, and the best NAA level was found 50 mg/l. Fattah *et al*., (1970); Hossain, (1976); Jahan, (2001), Kalita *et al*., (1995); Karim, (2005) found that productions of some crops were

increased by the apply of different growth regulators. For example, KNap and NAA were applied to some field crops.

There was also a proof that NAA had an optimistic effect on the dry matter accumulation in black gram (Saxena, 1994, Patel and Saxena, 1994), green gram (Kalita *et al*., 1995) and chickpeas (Karim, 2005). Productivity (number of pods) of some crops is increased using naphthalene acetic acid (NAA) such as cowpea (Deshai and Deore, 1985), lablab bean (Uddin *et al*., 1994), pigeon pea (Rao and Narayanan, 1998) and chickpeas (Karim, 2005). In addition, application of naphthalene acetic acid (NAA) induced increments in the 1000 seed weight in pea (Singh *et al*., 1972) and yield/plant (Karim 2005) in chickpea. Sultana *et al*., 2006 observed that NAA application at concentration 10 mg/l showed the best germination percentage and seedling growth. Similar results were also recorded by Shaikh *et al*., (2002) in onion. Also, Doddamani and Panchal (1989) found that NAA application at the same concentration (10 mg/l) increased the fruit length and thickness of chilli. Munsi and Sadhukhan, (1998) observed the greatest yield of chilli with NAA treatment at level 40 mg/l.

A foliar spray of NAA, KN, GA_3 and Ethrel increased number of fruits/plant; fruit weight and yield of chill (Lyngdon and Sanyal, 1992). Moreover, yield of chilli was increased by using different growth regulators such as NAA, TIBA, 2, 4-D and Ethephon (Indira *et al*., 1985, Doddamani *et al*., 1989, Singh *et al*., 1995). The increase in fruit set and plant production in long bean **(***Vigna unguiculata* var. *sesquipedalis* (L.) Verdcourt due to foliar spray of naphthalene acetic acid (NAA) was also reported by Resmi and Gopalakrishnan, (2004). Tongumpai, (1994) also reported that NAA application and water irrigation can encourage pod, seed setting and the weight of 100 seeds in many kinds of beans. Nevertheless, applied NAA at concentration 100 mg/l alone did not promote seed setting.

Water is an important aspect for plants at the flowering and seed setting stage (Kato, 1964; Westgate and Peterson, 1993). Manjunath Prasad *et al*., (2008) observed foliar spray of NAA at concentration 100 mg/l increased significantly seed yield. Applied NAA at rate 100 mg/l showed increased number of fruit/vine and fruit/yield. . It was significantly higher than GA_3 treatments at concentration 50 mg/l and control plant (Hilli *et al*., 2010). While Shantappa, (2004) observed that application of NAA at concentration 50 mg/l decreased sex ratio of bitter gourd, but it increased the germination and plant growth with high-quality seeds. These increases were possibly by reason of the influence of auxins on physiological changing like a sex ratio, increased fruit set, fruit weight and higher photosynthetic activity, synthesis and movement of metabolites from origin to sink points.

Application of naphthalene acetic acid (NAA) at concentration 15 mg/l caused an increase in vegetative growth, fruit set, seed yield, pod length, pod weight, pod number per unit area and pod number per plant in cowpea plants (Resmi and Gopalakrishnan, 2004). In addition, leaf area index, leaf chlorophyll content, photosynthetic rate and grain yield were increased by using NAA application at rate 30 mg/l (Kannan *et al*., 2003). Application of GA_3 at concentrations 25 and 50 mg/l and NAA at concentrations 20 and 40 mg/l increased the okra yield (Surendra *et al*., 2006). The growth regulator, naphthalene acetic acid (NAA) at various concentrations has an important effect on the root and bud growth of cucumber thus accelerates the growth. However, the root and bud growth was inhibited by the high concentration, especially 1mg and above (Tang *et al*., 2008). Tang *et al*., (2009) reported that application of naphthalene acetic acid (NAA) between 0.0001 to 10 mg/l concentrations caused a preventing effect on the elongation of cherry tomato root and inhibited a stem quality, cotyledon and the stitching of young stem. Nevertheless, the concentration of NAA at

0.0001-0.1 mg/l increased the weight of the cherry root and the rate of 0.0001 mg/l was the best concentration which had a positive effect on the elongation and weight of the seeding concurrently.

Tisserat *et al*., (1985) found that auxins could induce callus formation in tissue culture of plants, whilst naphthalene acetic acid (NAA) and indole acetic acid (IAA) encouraged extreme callus formation in water melon (Compton and Gray, 1993). Furthermore, Balogun *et al*., (2002) found that callus was formed and grew well when explants of fluted pumpkin were cultured on medium who containing both NAA and kinetin. Pretreatment of brinjal seeds for 24 hours with GA at rate 40 mg/l, IAA at rate 50 mg/l or NAA at rate 25 mg/l enhanced seed germination (Sadawarte and Gupta, 1968). Likewise, foliar spray of IAA at concentration 20 mg/l increased the yield of brinjal (Bisaria and Bhatnagar, 1978). Also, dipping of brinjal seedling roots in NAA at concentrations 0.1 and 0.2 mg/l for 24 hours were improved growth (Sambasiva *et al*., 1980). The eggplant seeds treated with GA, IAA and NAA at rate 10 mg/l had better germination than higher concentration or control (Gupta, 1971).

Plant growth regulators influenced the sex expression of cucumber plant (Jutamanee *et al*., 1993). Santos and Lopes, (1981) observed that NAA application at concentrations 100 to 200 mg/l reduced the male flower numbers. Application of NAA with concentrations from 1.0 to 100 mg/l at flowering stage increased the level of pollen germination (Ding and Zhang, 1988). Wu and Lin, (2002) reported that application of NAA with different concentrations (15, 20, 30 and 50 mg/l) at full bloom stage improved fruit quality. The best concentration of NAA application was 20 mg/l. But there was no significant effect on fruit quality when

application of NAA with same concentrations at young fruit stage. These result may be agreed with the fact that auxin and kinetin content enhanced after "pea-stage. Because of spraying NAA to the loquat fruits at pea-stage and replicating it one week later (Chaudhary *et al*., 1990) increased yield, single fruit weight, edible rates, TSS, and reducing sugar content. Schadler *et al*., (1994) observed that application NAA and other auxins to the pistil of rapid - cycling *Brassica Rapa* caused a formation of parthenocarpic fruit. Even so, NAA is reported to be more poisonous to plant than other (Nichell, 1982).

Iqbal *et al*., (2009) reported that application of NAA at variable concentrations to Guava (*Psadium guava* L.) red flesh cultivar decreased the fruit dropping, which resulted in an increase fruit yield. In addition, NAA treatment improved the fruit quality, and enhanced the pulp/seed ratio, TSS, total sugar and ascorbic acid contents in fruit. The best level of NAA was 45 mg/l where high concentration reduced the fruit quality. In addition, Dubay *et al*., (2002) reported that foliar sprayed of NAA to guava *(Allahabad Sufeda*) at concentration 250 mg/l increased yield and fruit quality. These results as well agree to those of Rajput *et al*., (1978) who observed an increase in fruit size, TSS, ascorbic acid and pectin contents of guava fruit at NAA concentration 40 and 80 mg/l. Several investigators also noted NAA application increased fruit size, fruit weight, TSS, total sugars and reduced acidity contents in mango and mandarin fruits, respectively (Maurya and Singh, 1981; Singh, 1980; Asi and Ali, 1970). Moreover, Eecher *et al*., (1981) found that application of NAA increased the soluble solids and acidity of apples. Kassem *et al*., (2010) found that foliar application of NAA and other chemicals to Costata persimmon trees at pea plus marble stages increased vegetative growth, fruit retention, tree yield, fruit weight, TSS, total sugars, reducing sugars, carotene and V.C contents and decreased fruit acidity and tannin contents. Likewise, Mohammad

Golam *et al*., (2008) reported that sprayed of NAA and other plant growth regulators with concentrations 25, 50 and 100 mg/l at three times (a day before, at anthesis and after anthesis) increased fruit size (length and diameter) and weight with the increase of concentration of plant growth regulators in Teasle gourd plant. Watkins and Cantliffe, (1980) reported that NAA application encouraged fruit set in *Cucumis sativus*. Rubasinghe, (2009) found that leaves of Chirita moonii grew in sand and coir dust (1:1) medium treated with NAA at rate 1000 mg/l increased the root fresh weight, number of roots and root length. While application of NAA at concentration 150 mg/l with the same medium was the best for root growth in soft wood cuttings of Chirita moonii.

Auxin applications to *Robinia pseudoacacia* stem cuttings increased the number of roots, root length, leaf number and the leaf area. However, root length and number of primary roots of rooted leaves reduced with high concentrations of hormones such as 2000 mg/l to 4000 mg/l, which can be killed the cells (Swamy *et al*., 2002). Additionally, Tchoundjeu and Leakey, (1998) found that high concentrations of auxins damaged the cells. Medhi and Borbora, (2002) reported that foliar spray of naphthalene acetic acid (NAA) at concentrations of 10 and 15 mg/l to *Phaseolus vulgaris* L. increased pod setting. El-shewy, (1999) indicated that sprayed of NAA at 50 mg/l and GA_3 at 50 mg/l at full bloom plus three months after first spray was most valuable treatments in reducing pre harvest fruit drop as well as fruit seed contents in guava. These beneficial effects of NAA and GA_3 application also were reported by Maurya and Singh in mango plant. Meanwhile, Dutta and Banik, 2007 found that application of NAA and GA to Sardar guava before flowering and three weeks after fruit setting increased fruit length, diameter, fruit weight and crop yield. Application of naphthalene acetic acid reduced the number of fruits currently this application increased the

average fruit diameter in loquat trees (Agust *et al*., 2000). In citrus plant, synthetic auxins increased the final fruit size but the effect of them, here was occurred by an enhancement of cell enlargement not cell division (El-Otmani *et al*., 1993; Agust *et al*., 1996).

Applied of NAA and GA_3 and promalin showed the increase in fruit yield of persimmon trees (Blumenfeld, 1986; El-Shaikh *et al*., 1999; Kabeel, 1999). Also, El-Shaikh *et al*., (1999) reported that foliar spray of GA_3 and NAA at various concentrations increased fruit weight. Harhash and Al-Obeed, (2007) found that sprayed date palm fruits (Barhee and Shahl) with NAA at the concentrations of 0, 50, 100, 150 and 200 mg/l increased bunch weight, fruit weight, height, diameter, size and flesh weight percentage. In addition, GA_3 increased moisture percentage of the fruit flesh. While NAA applications decreased TSS, total and reducing sugars in fruit juice, seed weight per fruit, acidity percentage and non reducing sugars of both cultivars were not affected. The best level of NNA for improving yield and fruit quality was 150 mg/l. But some worker fond that NAA treatment at rate 50 to 200 mg/l the valuable concentration for increasing fruit size, and weight in Zahdi and Sayer cvs. (Shabana *et al*., 1976); in Khenazi cv. (Shabana *et al*., 1998 and Aljuburi b *et al*., 2001), in Barhee cv. (Aljuburi a *et al*., 2001) and in Khadrawy cv. (Aljuburi *et al*., 2003) and Shahani cv. (Aboutalebi and Beharoznam, 2006). Stern *et al*., (2007) found that an increase in fruit size as a result of NAA application.

2.4 GIBBERELLINS

Another kind of hormone which found in plants is Gibberellic acid and there are around 100 gibberellins of this group are known. However, some of them have been found in fungi and some in higher plants (Plate 2.13). GA1 is more active than other gibberellins in the

promotion cell elongation but commercially GA_3 or mixture GA_4 plus GA_7 has been used in-plant culture (George, III, 2008). Now, crop yield is increased using different plant growth regulators, and they are widely used in horticulture for promoting plant growth in many plant species. One of the most important growth regulators is gibberellic acid, which used for promoting cell elongation, cell division (Akhtar *et al*., 2008). Japanese farmers found one disease attacked rice seedling and affected its growth. They observed occurrence of abnormal elongation in certain rice seedling where grew taller than normal one in the early season. These plants were unhealthy and sterile. Japan's people gave these conditions many names but *bakanae* (foolish seedling) were the most general term (Hori, 1898). This disease has many dangerous infections on the rice growth such as plants would grow excessively taller than the rest of the rice in the paddy, abnormal elongation, drop down and be unmarketable. Work on this physiology trouble in Japan happened just earlier than wars in the first half of the 20th century. Kurosawa, 1926 observed that a chemical in this fungus that caused the rice seedling grew so tall and toppling over. It is due to a fungus of the genus *Gibberella*. This substance called gibberellin (GA_3) or gibberellic acid (GA).

Gibberella fujikuroi is a fungus which was responsible for this disease. Yabuta (1935) isolated a substance which promoting rice root growth called gibberellin. After that Yabuta and Sumiki isolated another gibberellin from this fungus. Then the scientists in United States started their work after World War II. In 1950, scientists revived this work and extracted a variety of chemicals that draw out the growth response in rice seedlings. These are now known as gibberellins. Since that time, close to 100 slightly different gibberellins were identified chemically. It has been available commercially since the late 1950s. Gibberellins

have gibbane skeleton with specific biological properties (Plate 2.14) that let it chemically different from auxins.

Gibberellins' manufacture occurs mostly in the roots and young leaves. Little growth, in fact, arises in the root but mostly in the leaves and stems. Although there is the little effect on the root growth, the gibberellins encourage an enlargement in the stems and leaves. The seeds, young shoots, and roots of plants contain gibberellins, and they are also found in fungi. Inside the stems, the gibberellins stimulate cell division and cell elongation. Both auxin and gibberellins have to be active together to control stem growth. Most of the plant seeds have a high rate of gibberellins in the embryo.

* Gibberellin isolates by the embryo moves towards the aleurone layer, its target tissue located in the endosperm area of the seed (alongside the embryo).

* Gibberellin performs as the inducer, as its attendance lets the enzyme induction of amylase, which can break down starch into a sugar to be utilized in the embryo.

* Sugar is used in the plant to produce proteins and break out of dormancy.

Gibberellins have many effects on the plant which depends on the type of gibberellin and the species of the plant. Some of the physiological effects are given below.
(Davies, 1995; Mauseth, 1991; Raven, 1992; Salisbury and Ross, 1992).

1- Encourage stem elongation by stimulating cell division and elongation.
2- Stimulate bolting/flowering in response to long days.
3- Breaking of seed dormancy and tubers:

4- Stimulate enzyme production (a-amylase) in germination cereal grains for mobilization of seed reserves.

5- Promote the production of male flowers in dioecious flowers.

6- Induce parthenocarpic fruits.

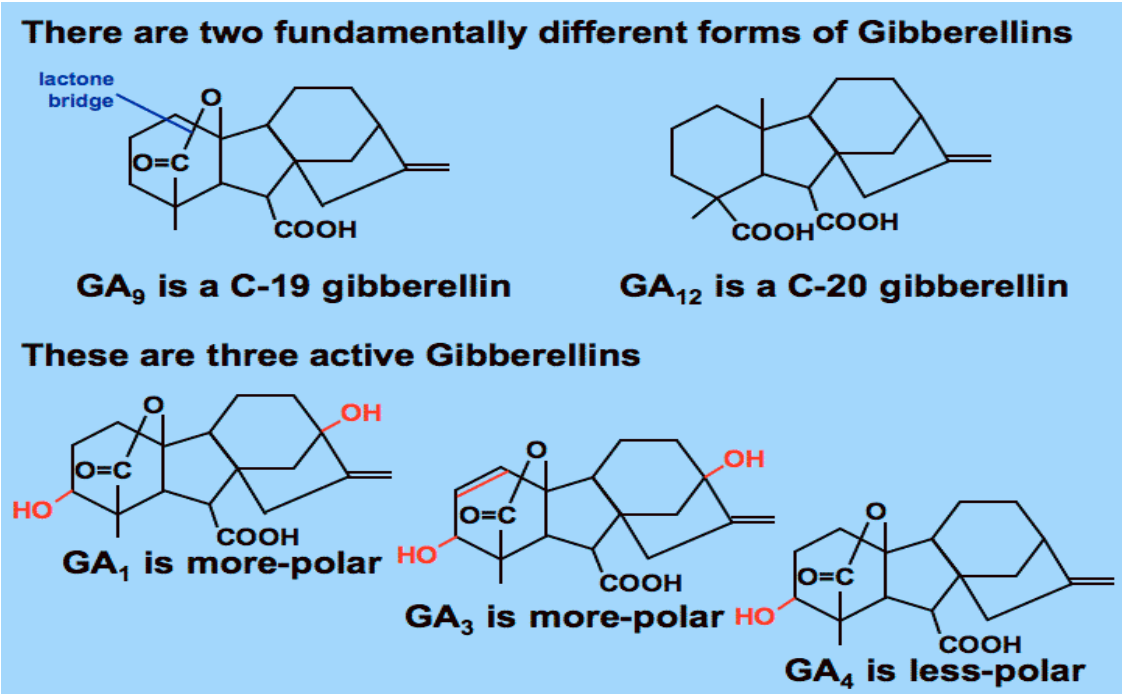

Plate 2.13. Some common gibberellins.

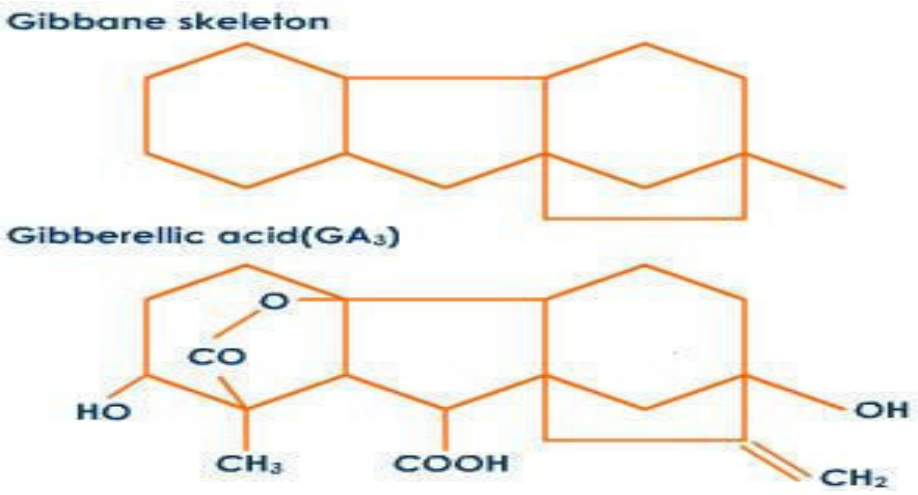

Plate 2.14. Structures of Gibbane Skeleton and Gibberellic Acid (GA_3)

2.4.1 Effect of gibberellic acid on plants

Gibberellins (GAs) are a family of plant hormones that have many responses in plants, through seed germination to senescence. The mainly generally available compound is GA_3 or gibberellic acid, which tempts stem and internode elongation, seed germination, enzyme production during germination and fruit setting and growth (Davies, 1995). Plant growth regulators (PGRs) are also used with the aim of manage vegetative growth (Latimer, 1991). Cowpea seeds soaking with GA_3 at concentration 10 mg/l had a significant effect on rate germination, seedling growth, and plant height.

In addition, Levy *et al*., (1986) indicated that foliar application of GA_3 at rate 280 mg/l encouraged flowering, number and weight of capsules per plant of *Papaver*. Furthermore, Pereira and Maeda, (1986) observed that GA_3 application had a positive effect on germination of *Vitis vinifera*. Fahmy *et al*., (1987) found that GA_3 application increased germination of Kenaf whereas it decreased it in Roselle. Treatment with gibberellic acid at

different concentrations (25, 50 and 75 mg/l) resulted in increased leaf number and fresh fruit yield of cucumber plant under green house conditions (Batlang *et al*., 2006). Also, they found that the increase in fresh fruit yield of cucumber is related to GA_3 concentration. They suggested that application of GA_3 and Benzyladenine plus GA_{4+7} were usefully treatments to improve the cucumber growth under greenhouse conditions.

Brock and Cleland, (1990); Keyes, (1990) found that gibberellin application is enhanced cell enlargement by increasing cell wall of the plant extensibility. Manjunath Prasad *et al*., (2008) stated that gibberellic acid; naphthalene acetic acid and ethrel treatments induced positive effects on crop growth, fruit yield, seed yield and seed quality aspects in cucurbitaceous crops. Also, similar results had been achieved by Goudappalavar (2000) in tomato, Singh and Lal (1995) and Balaraj (1999) in chilli. Ouzounidou *et al*., (2010) reported that sprayed of GA_3 at level 100 mg/l and other chemicals enhanced flowering, improved the vegetative characteristics of *Capsicum annuum* L. Additionally; GA_3 treatment increased the quantum yield of primary photochemistry (Fv/Fm). They also observed that spray application of gibberellic acid two times at two weeks intervals and three weeks after seed germination, significantly the fruit yield and reaches suitable quality of *Capsicum.* Davies, (1995) informed that the most commonly available PGRs was gibberellic acid, which stimulated stem and internode elongation, seed germination, enzyme production through germination and fruit setting and development. However, plant growth regulators are as well used to organize vegetative growth (Latimer, 1991; Ouzounidou *et al*., 2008). Application of GA_3 significantly increased ascorbic acid content of chilli (Chaudhary *et al*., 2006). Also, spray of GA_3 increased the soluble solids and ascorbic acid of peppers. Gonzalez-Rossia *et al*., (2007) observed that application of GA_3 increased quality characteristics of peaches and nectarines.

Akhtar *et al*., (2008) found that presowing treatment of spinach seeds with GA_3 at 10 ppm had a remarkable beneficial effect on the number of leaves and germination percentage. Gibberellic acid is one of most significant growth motivating substance utilized for encouraging cell elongation, cell division and therefore, to promote growth and improvement of many plant species. Application of GA_3 at low concentration a little increased germination in three different species but at high concentration of GA_3 inhibited germination (Wang *et al*., 2000). Effect of gibberellic acid on germination of *Pediculari's* species (Ai-Rong, 2007), *Eremurus spectabilis* (Rahmanpour *et al*., 2005), *Rhodiola rosea* (Aiello and Fusani, 2004) and *Pterocarpus angolensis* (Chisha – Kasuma, 2007) have been studied. Sarkar *et al*., (2002) found that gibberellic acid application promoted plant height, number of branches, number of leaves, leaf area per plant, number of flowers, number of pods, percentage of fruit set, number of seed per plant, seed yield per plant, 100-seeds weight and seed yield at concentration 100 mg/l in soybean, in groundnut and sorghum (Lee, 1990; Shinde *et al*., 1989). Additionally, seed yield per plant in rice was increased by application of GA_3 at 100 mg/l (Awan and Alizai, 1989), soybean (Deotale *et al*., 1998; Maske *et al*., 1998), bell pepper (Abdul *et al*., 1988) and onion (Hore *et al*., 1988).

Gibberellic acid has been used to stimulate stem elongation (Harrington *et al*., 1996), increased dry matter accumulation (Hore *et al*., 1988) and enhanced total yield (Deotale *et al*., 1998; Maske *et al*., 1998). Moreover, GA_3 has been used experimentally to increase plant height was reported earlier in soybean (Deotale *et al*., 1998), okra (Kumer *et al*., 1996), sesame (Sontakey *et al*., 1991), rice (Awan and Alizai, 1989) and groundnut (Lee, 1990). Awan and Alizai, (1989); Lee, (1990); Sontakey *et al*., (1991); Deotale *et al*., 1998) observed that GA_3 application improved to enhance the number of branches/plant in many crops.

It researches on the effect of plant growth regulators in solanaceous fruit and vegetable crops have exposed that the application of some of the PGRs has been found useful in reducing the flower and fruit drops thus enhancing production of chilli per unit area and per unit time. A number of researches were performed with aspire of removing the inhibitory result of high-temperature stress on germination, by using variety of a growth regulators. It has been identified for a long time that gibberellins (Biddington and Thomas, 1978, Biddington *et al*., 1980), cytokinins (Kaufmann and Ross 1970, Keys *et al*., 1975) decreased the undesirable effects of high-temperature stress during seed germination. Just, three of the single applications gibberellic acid, kinetin and 24-epibrassinolide might improve the effects of high temperature on germination of barley seeds. Ouzounidoul *et al*., (2008) found that GA_3 application enhanced the total stem length in addition to the elongation of the first internode and leaf, and improved ascorbic acid content of *Cucumis melo* L. plants. Moreover, they found that GA_3 application increased the Fv/Fo ratio, increased fruit diameter. Even so, it did not show any effect on fructose, glucose and the Soluble Solids of melon fruits. Exogenous GA_3 provided stimulation to cell elongation and/or cell division; chlorophyll fluorescence indices supply direct information on functionality and the effectiveness of photosynthesis (Lichtenthaler *et al*., 2005).

Plant growth regulators are used commercially for improving the productivity and quality of a number of crop plants (Janick, 1979; King and Evans, 2003; Mukherjee and Prabhakar, 1980; Singh, 1995). GA_3 application encouraged cell enlargement and cell division (Arteca, 1996; Liu *et al*., 1976 and Moore, 1989) significant processes that increase plant height and leaf area. Masroor *et al*., (2006) observed that foliar spray of GA_3 at four different

concentrations (0, 10^{-8}, 10^{-6} and 10^{-4} mg/l) in pots trial increased plant height, leaf area, leaf P content, fruit number, fruit yield and fruit lycopene content. Gibberellins contribute in regulation of many growth developments in some crops. (Naeem *et al*., 2001; Emongor, 2007 and Shibairo *et al*., 2006) found that GA_3 is important for regulating stem elongation (Sakamoto *et al*., 2004 and Sun, 2004). Haba *et al*., (1985), Khafagi *et al*., (1986), Kumar and Neelakandan, (1992); Maske *et al*., (1997) and Yamaguchi and Kamiya, 2000 reported that gibberellins (GA_3) played an important role in many characteristics of plant growth and development, such as seed germination, stem elongation and flower development. Whereas applied at the pre-blooming stage, gibberellins (Gas) reduced the number of flowers and fruit set, possibly by increasing vegetative mass which, in order, shared the photo assimilates with the fruit (Birnberg and Brenner, 1987). This hypothesis is also constant by King *et al*., (2000), who found that larger stem growth occurred in *Fuschia hibrida* and *Pharbitis nil*, resulting in the inhibition of flowering. An increase in the number of leaves was demonstrated after treatment with GA_3 by Harb (1992) for *Vicia faba*, This displays that contrasts between different species should not be made, because the response could be related moreover, to the different application techniques and rates (King *et al*., 2000). The enhancement in-plant stem growth as a reaction to GA_3 happened as a consequence of cell elongation (El Fouly *et al*., 1988; Tanimoto, 1990) the increase in leaf area was recognized to cell elongation.

Stuart, (1971) found that GA_3 may become one of the necessary components of media for culturing cells at low concentrations. High concentration of GA_3 has a significant effect for inducing caulls cells (Schroeder and Spector, 1957; Murashige and Skoog, 1962; Mehra and Mehra, 1972; Altman and Goren, 1974; Beasley, 1977; Gautam *et al*., 1983) and enhanced

the growth of caulls in mixture with auxin and low concentrations of cytokinin (Engelke *et al*., 1973). Application of GA_3 at level 2 mg/l inhibited callus growth of *Solanum xanthocarpum* B (Rao and Narayanaswamy, 1968) while GA_3 application enhanced the growth of cells in suspension cultures (Davidonis, 1990).

Gibberellic acid (GA_3) is known to stimulate the synthesis of a-amylase and hydrolysis of starch in rice seeds (Palmiano and Juliano, 1972). Lin and Kao, (1995) observed that gibberellic acid decreased NaCl inhibition of a-amylase activity under salt stress. Kim *et al*., (2006) also found that presowing of rice seeds in GA_3 at level 10 mg/l increased total soluble and reducing sugars in endosperms and increased the germination progressed under low NaCl stress. In addition, Agakishiev, (1964); and Zhao *et al*., (1986) reported that GA_3 application increased the growth of cotton and some halophytes in saline situation. Also, GA_3 frustrated the influence of NaCl on the carbohydrate metabolism in leaves of Pennisetum typhoides (Huber *et al*., 1974). Kaur *et al*., (1998) reported that adding of exogenous gibberellic acid motivated an increase in germination and seedling growth by enhancing the availability of endogenous gibberellic acid. Moreover, gibberellic acid application enhanced leave number per plant of onion (Shishido and Saito, 1984), and increased in leaf length (Singh *et al*., 1983; Salah and Abd, 1989). In this respect, Khurshid, (1992) observed that GA_3 application reduced leaf number in grapevine. Gibberellic acid at concentration 100 mg/l improved yield in rice (Verma and Singh, 1979; Awan and Alizai, 1989), soybean (Deotale *et al*., 1998), onion (Hore *et al*., 1998) and okra (Rahman *et al*., 1994). Mathur, (1971) found that spray of GA_3 increased bulb diameter, and increased length and weight of okra pods at all concentrations (Rahman *et al*., 1994).

Agboola and Adedire, (1998); Fasidi *et al*., (2000) and Irish, (1996) stated that plant growth regulators stimulated seed germination and plantlet physiology (shoot growth, flowering, floral development and fruit set. Application of GA_3 and IAA improved shoot elongation in savannah legume*; Parkia biglobossa*, *Senna siamea*, *Albizia lebbeck* and *Prosopis africana* (Ebofin *et al*., 2003). Moreover, Ingrame *et al*., (1986) and Davies (1995) observed that GA_3 application regulated height in pea (*Pisum sativum*). Harrington *et al*., (1996) reported that GA_3 encouraged stem growth by promoting cell division and elongation. In addition, Bekheta (2004) informed that foliar application of gibberellic acid induced an increase in plant height of wheat plants. Furthermore, El- Beheidi *et al*., (1991); Shalaby and Abdel-Halim, (1995); Abd El-Fattah, (1997); Bekheta, (2000); El –Kady, (2002) and Mahgoub *et al*., (2006) found that application of kinetin, GA uniconazole on faba bean increased the number of leaves/ plant.

Plant growth regulators have optimistic effects on plant height, number of branches, and leaf area in addition to the number of pods per plant. Several researchers obtained a positive effect of bioregulators on various plants (El-Beheidi *et al*., 1991 El-Quesni *et al*., 1992) using CCC on faba bean; Shalaby and Ahmad, (1994) IAA on the lentil; Shalaby and Abdel-Halim (1995) kinetin and GA on faba bean; Bekheta, (2000) and El-Kady (2002) uniconazole on faba bean and wheat plants correspondingly; Bekheta, (2004) and Mahgoub *et al*., (2006) paclobutrazole on wheat and *Calendula officinalis* L. respectively. Similar results are in agreement with those obtained by many investigators (Reddy *et al*., 1999; Xu and Taylor, 1992, Bekheta 2004 and Bekheta *et al*., 2006) they reported that growth regulator treatments enhanced chlorophyll content with can be relatively well explained by the preferred inhibition of cell extension by growth regulators. Due to inhibition of cell extension, there are more

cells per leaf blade and per fresh matter. Thus even at constant chlorophyll content/cell, chlorophyll content/leaf blade or /fresh matter would be increased.

Foliar application of *Vicia faba* plants with different plant growth regulators at all the used levels laded to clear increase in the endogenous content of mineral ions (Ca, K and Mg). Foliar application of GA_3 at concentration 50 mg/l was increased pod length of faba bean plant (Abd-El Fattah *et al*., 1997). Auxins, gibberellins (GA_3) and kinetin improved plant growth and development processes (Frankenberger and Arshad, 1995). As well Karssen *et al*., (1989); Hayashi, (1940); Wittwer and Bukovak, (1957) reported that gibberellic acid (GA_3) assisted cell elongation in different organs and tissues during plant growth and development. Gianfagna, (1995) informed that GAs application promoted the growth of a variety of fruit crops. It also increased sugar yield in sugarcane, and to accelerate the barley-malting process in the beer-brewing industry. It repeated spraying with GA_3 to table grabs increased both rachis length and fruit size. Moreover, two to three supplementary applications of GA_3 through fruit growth were thought to enhance berry size by encouraging the import of carbohydrates into the developing fruit.

Gibberellic acid was also used to enhance cherry production; Sweet and Bing cherries. Application of GA_3 to tart cherries increased yield through enhanced bearing. Bukovac, (1963); Bukovac and Nakagawa, (1967); Davison, (1960); Nakagawa *et al*., (1967) found that application of gibberellin (GA_3) and the combination of gibberellins with cytokinins (Bangerth and Schroder, 1994; Williams and Letham, 1969) encouraged parthenocarpic fruiting in apple. Single and combined applications of gibberellic acid induced parthenocarpy (Bukovac, 1963; Bukovac *et al*., 1967; Davison, 1960; Nakagawa *et al*., 1967). Application

of gibberellins before flowering induced parthenocarpic fruits. Gibberellins that are present in the receptacle and/or ovary before flowering might be essential to stimulate parthenocarpy; they play a slight role in fruit development after flowering. However, Kowalska, (2008) reported that gibberellin application improved parthenocarpic fruits. In this respect, Abd-Alaai *et al*., (1982) observed that application of GA_3 between 25 to 100 mg/l concentrations resulted in formation of seedless date palm. Also, Single or double application of GA_3 at concentrations 50 or 100 mg/l to unpollinated spadices formed seedless fruit, but they were lighter in weight, longer and thinner than seed fruits (Abou Aziz *et al*., 1982). The fruit physical characteristics were not affected significantly by the GA_3 treatment when it applied to pollinated fruits (Hussein *et al*., 1974).

The PGRs played a major role in the growth and development of plants. Several investigations have been reported concerning the effect of applications of plant growth regulators on plant physiology and nutriology (Pan and Li, 1999; Amarjit, 2000). The PGRs both synthetic and natural play a major role in the various types of plant growth and development (Leopold, 1962; Weintraub and Norman, 1949). PGRs, used as trace signal molecule in plants, had the very important impact in regulating all kinds of growth processes and environmental reactions, and in the meantime, made great contribution to the agricultural chemical control of crops, fruits and vegetables (Xu and Li, 2006). PGRs mostly organize the plant physiological processes such as growth and development, organ formation and so on. The dynamic modifies of endogenous hormone levels during the stage of fruit growth have been studied by many investigators. Several results showed that GA could promote an increase of grape fruit grains (Kim, 1991; Retamales, 1993).

Soliman, (2006) carried out an experiment that sprayed of GA_3 at different concentrations (0, 50,100 and 150 mg/l) to Sakkoty date palm two seasons. The higher concentrations of GA_3 application to pollinated fruits and 50 days after full bloom increased average fruit weigh, flesh, weight, fruit length, fruit diameter and fruit moisture content percentage in both seasons. Meanwhile, application of GA_3 on various concentrations caused a minor reduction the total soluble solids percentage, reducing sugars percentage and total sugars percentage and increase of total acidity percentage during the two seasons. The heights yield with best fruit quality and fruit characteristics were observed in the concentrations 100 and 150 mg/l. Abo-Aziz *et al*., (1982); El-Hodairi *et al*., (1991) and Abo-El-Ez *et al*., (2002) observed that different concentrations of GA_3 increased the fruit weight of date palm. Furthermore, it was found that, some physical properties that were determined in this study extensively increased by enhancing the concentration gibberellic acid. In addition, Abo-El-Ez *et al*., (2002) observed that GA_3 treatment reduced the total soluble solids (TSS).

Wiggans and Martin, (1961) found that pretreatment of pecan seeds with gibberellic acid (GA) in up to 5000 mg/l tended to cause earlier germination and high germination rates comparing to untreated seeds. Also, Wiggans, (1962) observed that presowing treatment of 'Westren' pecans for 48 hours in 100 mg/l GA_3 tended to encourage earlier germination and seedling emergence than 100 mg/l IAA mg/l application. Abdel-Hady *et al*., (2008) found that seed germination rate of *Atropa belladonna* L. increased at higher concentrations of GA. Moreover, Ruminska *et al*., (1978) found similar results with seven species of seeds, but the best results were achieved with Lavandula *Vera* and *Atropa belladonna*.

Mukhtar, (2008) found that foliar application of GA_3, auxin and 15% coconut milk with two concentrations (50 mg/l, 100 mg/l) to *Hibiscus sabdariffa* L. (Red sorrel) considerably increased plant height, stem girth, leaf development and chlorophyll content of the vegetable with higher rates recorded in application with 100ppm GA_3, 100ppm IAA and 15% coconut milk. Additionally, application of GA_3 at 100 mg/l and 15% coconut milk had a greater effect on increase carbohydrate and vitamins A, B6 and C contents of the vegetable. Whereas GA_3 application at 100 mg/l increased sodium, copper and zinc rates, 15% coconut milk at 100 mg/l increased phosphorus and potassium levels. Thomas, (1976) observed that gibberellic acid had been used to encourage stem and petiole expansion in rhubarb, celery and water cress. Treatments of gibberellic acid, 4-chloroindole and 6-benzyl amino purine to the standard petal and calyx of *Vicia faba* var. major was found to significantly increase pod set (Rylott *et al*., 1990). In another hand, application of *Vicia faba* cv. Troy reproductive formation with indole-3-acetic acid, GA3 or 6-benzyl amino purine resulted in the increased fruit number (Clifford et, al., 1992). Currah and Thomas, (1979) reported that spraying of GA_3 on carrot plants with concentration 100 mg/l enhanced plant height. Also, application of GA_3 improved the growth of bean and leaf length of lettuce (Biddington *et al*., 1987). Chakrabarti and Chakrabarti (2003) observed that gibberellin treatment avoided the effect of unfavorable environmental conditions. Application of plant growth increased the saline tolerance of many crop plants (Haroun *et al*., 1991; Hoque and Haque, 2002). Exogenous GA_3 had also been shown to ease the effects of salt stress on water-use efficiency (Aldesuquy and Ibrahim, 2001). Das Gupta *et al*., (1994) observed that foliar spray of plant growth regulators such as IAA and GA assisted the plant to reinstate retardation in water content in Mungbean plants subjected to water stress. Likewise, Chakrabarti and Chakrabarti (2002) informed that GA_3 application avoided undesirable effects of water stress to Mungbean

plants. The roles of plant growth regulators avoid the dangerous effects of salinity on growth possibly due to modify in the endogenous growth regulators who affect plant water balance.

Gibberellins and auxins organize many performance functions in plants, which are active as chemical messengers manipulating many patterns of plant development. These encourage cell division and cell elongation and control enzyme secretions. Seed germination and seedling growth can be influenced by various concentrations of growth regulators such as GA_3 and IAA. GA_3 application was used to control cracking of pomegranate fruit (Sepahi, 1986) and litchi (Sharma *et al*., 1986) and apple russeting (Taylor and Knight, 1986) and to inhibit flowering of *Prunus* species (Coneva and Cline, 2006; Lenahan *et al*., 2006). Proebsting *et al*., (1973); Facteau, (1986); Facteau *et al*., (1985); Looney, (1985); Facteau (1986); Sive and Resnizky, (1988); Kupferman, (1989); Choi *et al*., (2002); Kappel and MacDonald (2002); Horvitz *et al*., (2003); Clayton *et al*., (2006); Özkaya *et al*., (2006) reported that application of GA_3 had been generally used in commercial horticulture trees since they have consistently been shown to increase fruit size and firmness of cherry. In this respect, spray of GA_3 to cultivars Merton Premier, Bing, and Dawson delayed harvest, increased fruit firmness, weight, and soluble solids, but had no influence on fruit cracking. While GA_3 treatment increased fine fiber yield and improved fiber fineness, it decreased chlorophyll content, stem diameter, flowering and boll production over untreated controls (Ayala-Silva *et al*., 2004). Nixon, (1959) found that GA_3 application to different cultivars of date palm resulted in an increase length of the fruits but the effect of GA_3 on the fruit length was variable. Application of gibberellic acid at 20, 50 and 100 mg/l concentration to pollinate bunches caused an increase in fruit size (Ketchie, 1967). Wanyama et. al., (2006) obtained that application GA_3 enhanced branching, flower bud formation and fruiting. GA_3 application

at 100 mg/l one week after transplanting the seedling showed the greatest growth, branches number, fruit production and plant height. GA_3 application also increased the growth of fenugreek (Khan and Rashid, 1983; Deore and Bharud, 1990). In this respect, several researchers found that GA_3 treatment improved plant production (Gozales, 1978; Rao *et al*., 1981; Joshi and Singh, 1982; Khan *et al*., 1983; Jain and Agrawal, 1988).

Several studies have found that application of gibberellins induced early anthesis in strawberry (Porlingis and Boynton 1961) and coffee (Schuch *et al*., 1990). Porlingis *et al*., (1961); Guardiola *et al*., (1977) and Lord and Eckard, (1987) stated that the stimulatory (promotive effect) and inhibitory (inhibitory effect) influence of GA_3 on flowering depend on plant species, concentration and time of application. GA_3 treatment at the rate of 30, 60 and 90 mg/l increased cowpea plant height, first node height, leaf area, leaf number/plant, nodulation, plant dry matter accumulation, pod length, pod number/plant, seed number/pod and 100 seed weight (Emongor, 2007). However, gibberellins (GA_3) were applied in some cowpea varieties to modify growth and improvement. Studies on the effect of gibberellic acid in Arabidopsis have revealed that the application of GA_3 has been found effective in inducing early flowering and flower morphology (Richards *et al*., 2001). Gibberellins enlarge very young leaves by enhancing the cell number while enlargement of older leaf results from cell enlargement (Briant, 1974; Goodwin, 1978). In addition, Pharis *et al*., (1985) stated that gibberellins enhance the growth of floral organs as well as corolla in many species.

Hollingsworth, (1981) reported that vegetables are necessary in the diet since they present plant fiber, mineral elements, vitamins, carbohydrates and proteins. Okra is one such important vegetable belongs to family Malvaceae and is cultivated for its immature fruits,

which is eaten either as a cooked vegetable. It offers some nutritional elements such as calcium, potassium and other mineral matters with medium percentages of (Riboflavin, Thiamin, Vitamin-C and Vitamin-A) (Matlob et. al., 1989). Moreover, okra can be used in food processing, oil (mature seeds have about 20% edible oil), paper and sugar manufacturing (Reddy et. al., 1997).

Abelmoschus esculentus L. is a native of Africa and is a hot season annual which is adapted to a broad variety of soil types. There is an urgent demand to increase up the production and fruit quality of this vegetable. Plant growth regulators have been suggested as possible tools for food production (Belakbir *et al*., 1998; Olaiya, 2006). Plant growth regulators are the new generation of agrochemicals to control plant physiological and biochemical developments in plant, when used them with small concentration, enhance the natural growth right from seed germination to senescence in crop plants such as control of dormancy, organ size, crop development, flowering and fruit set and regulation of chemical composition of plants (Nickell, 1978). However, Plant growth regulators play an important role in a host of physiological processes of plant is well-known in the literature.

Some growth regulators influence mainly vegetative growth; others manipulate the fruit only; still others can stimulate changes in both vegetative and fruiting parts. Martinelli *et al*., (2009) stated that parthenocarpy is a desirable trait for many commercially grown fruits if undesirable changes to structure, flavor, or nutrition can be avoided, and seedless fruits are more suitable than seeded fruits to customers. Varoquaux *et al*., 2000 informed that replacing seeds and seed cavities with edible fruit tissue is desirable. Okra seeds used to make some people so nauseated. The plant growth regulators enhanced fruit set on tomatoes and some

other vegetable (cucumber, tomato, bottle gourd, brinjal, *Cucurbita*, watermelon) (Avery and Johnson, 1974; Homan, 1964; Elassar *et al*., 1974; Miyazaki, 1965; Terada and Masuda, 1941), and can induce growth of seedless figs (Julian and Blondeau, 1949; Stewart and Condit, 1948). Furthermore, Choudhury and Pathak, (1959); Elassar *et al*., (1974); Kulkarni and Rameshwar, (1978); Yu, (1999); Hayata *et al*., (2000) found that gibberellins and cytokinins induced formation of parthenocarpic fruits.

In plants, many behavioral mold and functions are restricted by hormones. These are "chemical messengers" manipulate many patterns of plant improvement. Plant growth regulators are natural substance (produced by plant) that acts to organize plant activities. The manipulation of growth and increasing productivity and quality of vegetables is the base for most plant researches. Auxin hormone is formed by the tip of a coleoptile, i.e. the first leaf of a plant. Auxin arises in small rates at the growing tips, and they act followed by translocation basipetally. Auxin plays an important role in organ differentiation. When a cut surface of a young stem is treated with a combination of high percentage of auxin, an undifferentiated mass of callus tissues is developed. Auxin also assists in fruiting. It enhances the ovarian tissue to form fruit. Abscission of leaves, flowers and fruits are controlled by auxin. Arditti, (1979) reported that the earliest plant growth hormones have been used with seed culture were auxins. The most common have been used with these cases were mostly NAA, IAA and IBA. They improved the germination and seedling growth. Furthermore, gibberellins are there in developed seedlings and also richly available in the growing tissues such as growing cotyledon or leaf. Gibberellins are the natural growth regulating systems in higher plants. They are involved in the phenomena of dormancy, flowering, and fruiting. It causes the formation of male flowers whereas its lower concentration favors the formation of female

flowers. IAA, GA_3 and NAA are known to be the most common growth regulators. Though there are some other hormones as well, but in the present study, the main concern is with IAA NAA and GA_3.

Plant growth regulators can be applied to plants by different methods. The common methods are root, foliar and presowing seed treatments. In addition, injection method can be done directly to the trunk of woody ornamental (Gent, 1997; Smith, 1998) and fruit (George and Nissen, 2002) crops. Shigetoshi, (1981) found that injection method of hormone application was a useful as a method of bioassay for improving the thickening growth of radishes. Nevertheless, foliar application of plant growth regulators has a low effect in some parts of the plant and spraying technique is related with drift of PGRs (Davis *et al*., 1988; Cramer and Bridgen, 1998). Common problem of using soil and foliar applications is highly concentrated solutions (up to 7,500 mg/l) of plant growth regulators may be needed with these applications (Orzolek and Kaplan, 1988). From this information a mentioned above, crop production will have a high remains level of growth regulators (Davis *et al*., 1988). According to this residues of growth regulators in the edible parts of crops will be a harmful to human health, which limited the usage of plant growth regulators to vegetables (Davis *et al*., 1988). On another hand, presowing seed treatment is another method, which can be used for applying plant growth regulators to plants (Fletcher *et al*., 2000). This method has some advantage such as a smaller amount of active ingredient, lower drift of product, and ease of application (Pasian and Bennett, 1999; Fletcher *et al*., 2000). It was also shown that the treating seeds with growth regulators induced change in plant growth and stress resistance (Fletcher *et al*., 2000). Some disadvantages results from treating seeds with growth regulators include decrease and interruption in seedling growth (Giba *et al*., 1993; Pill and Gunter, 2001).

CHAPTER 3
MATERIALS AND METHODS

3.1 FLOWER INJECTION METHOD APPLICATION OF PLANT GROWTH REGULATORS AT DIFFERENT CONCENTRATIONS.

3.1.1 Experiment 1

Effect of the Flower injection method on okra growth and development by using Indole acetic acid (IAA); Naphthalene acetic acid (NAA) and Gibberellic acid (GA_3)

3.1.1.1 Study site and Climatic information

The present investigation was carried out in a commercial farm in Banting, Selangor, Malaysia. The experiment field was located at 1º28´ N latitude and 111º20´E longitude at the height of 44.81 m above the sea level. The area of this study had a hot and humid tropical weather. The soil in this field was peat with a mean pH of 6.6. Experiments were run for 2 years (2009-2011).

3.1.1.2 Plant materials

The seeds of local variety of *Abelmoschus esculentus* were sown in the experimental plot in Banting. The seeds were soaked in distilled water for 24 hours after which they were spread on moist filter paper in Petri-dish. The Petri-dish was kept in dark cupboard at room temperature of 30 ºC. Okra seeds were sown directly into the soil by hand, and plots were irrigated when necessary. The whole area was divided into fifteen blocks and each block into 20 unit plots. The size of the unit plot was 1 x 1 m². The seeds were shown in rows made by hand plough. The gaps where seeds failed to germinate were filled up within two 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 done uniformly (Plate 3.1).

3.1.1.3 Preparation of IAA, NAA and GA_3 at selected concentrations

Freshly prepared aqueous solutions of IAA; NAA and GA_3: The concentrations of the IAA; NAA and GA3 treatments were 0 (water), 25, 50, 100 and 200 mg/l. The IAA NAA and GA_3 were dissolved in 2 ml of 1% ethanol to make desired concentration. Each concentration of IAA NAA and GA_3 was added with distilled water to make 100 ml of solutions. The control was 100 ml of water mixed with 2 ml of 1% ethanol.

3.1.1.4 Application of IAA; NAA and GA_3

One ml of the various concentrations of IAA; NAA and GA_3 were applied to the flower by injecting before anthesis with a needle for a surgical purpose (Plate 3.2). Four (4) flowers were selected randomly for each replication.

Plate 3.1. Photo shows okra plants field at Banting, Selangor.

Plate 3.2. Photo shows flower injection technique before anthesis.

3.1.1.5 Data collection and analysis

Data were recorded considering following parameters:

3.1.1.5.1 Pod setting

Pod setting percentage was calculated using the following formula

$$\text{Pod setting (\%)} = \frac{\text{Total number of pods}}{\text{Total numbers of treated flowers}} \times 100$$

3.1.1.5.2 Pod parameters

- Green pod length (cm)
- Green pod diameter (cm)
- Pod size (cm^2)

Pod size was determined by measuring the length and pod diameter per treatment with a Vernier caliper.

3.1.1.5.3 Single pod weight (Average)

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

3.1.1.5.4 Seed production

For the determination of healthy seeds from treated flowers, the number of health seeds and aborted seeds was counted at dry stage.

• Healthy seeds/pod (%)

Healthy seeds percentage was determined using following formula

$$\text{Healthy seeds (\%)} = \frac{\text{Total numbers of healthy seeds}}{\text{Total number of seeds}} \times 100$$

• Aborted seeds/pod (%)

Aborted seed percentage was calculated by following formula

$$\text{Aborted seeds (\%)} = \frac{\text{Total numbers of aborted seeds}}{\text{Total number of seeds}} \times 100$$

3.1.1.5.5 Total soluble solid (TSS)

A small fraction of the homogenous mixture (??) was centrifuged at 4000 ×g for 10 min and the clear supernatant was analyzed for TSS. The total TSS of pods was examined using a hand refractormeter an Atago 8469 (Atago Co. LTD., Tokyo, Japan and expressed as º Brix).

3.1.1.5.6 Measurement of Vitamin C

Vitamin C is an antioxidant which is important for human nutrition. Ascorbic acid was measured by redox titration. However, this method determined for the vitamin C concentration in sample by a redox titration with potassium iodate in the presence of potassium iodide. The ascorbic acid had been oxidised, the excess iodine was free of reaction with the starch indicator and formed the blue-black starch-iodine complex (Figure 17). This

was the endpoint of the titration. However, this method was more reliable as the potassium iodate solution was more stable than iodine as a primary standard. Since Iodine regularly reacts with oxygen in the air to form a mixture of IO, IO_2^-, and IO_3^- ions. Therefore, the concentrations in the solution modify over time. Potassium iodate (IO3) doesn't oxidize in air, so keeps its concentration.

Preparation of solutions:

• Starch indicator solution (1%): 0.50 g of soluble starch was weighed and added it to 100 mL of boiling water in a 150 mL conical flask. Then the solution was stirred to dissolve and cool down before using.

• Iodine solution:

1- 5g of Potassium iodide (KI) and 0.268g of Potassium iodate (KIO3) were dissolved in 200 ml of distilled water. After that 30 ml of 3 M sulfuric acid was added.

2- This solution was poured into a 500 ml graduted cylinder and diluted it to a final volume of 500ml with distilled water

3- The solution was mixed then solution was transferred to a 600 ml beaker.

• Vitamin C standard solution:

1- 0.250g of vitamin C was dissolved in 100 ml distilled water.

2- Then the solution was diluted to 250 ml with distilled water in a volumetric flask.

• standardizing solution:

1- 25 ml of Vitamin C was added standard solution to a 125 ml Erlenmeyer flask.

2- 10 drops of 1% starch solution were added and mixed.

3- The buret was rinsed with a small volume of the iodine solution and then was filled it. The initial volume was recorded.

4- The solution was titrated until the endpoint was reached.

5- The final volume of iodine solution was recorded.

The required volume was, (starting volume - the final volume).

6- The titration was repeated at least twice more.

Okra pod sample preparation: A 100 g of okra pod was cut into small pieces and was ground in a mixer. Then it was added to 10 mL of distilled water several times while grinding the sample and the liquid extract was poured off into a 100 mL volumetric flask. Finally, filtered ground okra pod was strained through cheese cloth then washed with 10 mL of water and collected all filtrate and washed in the volumetric flask. After filtration of the homogenate through cheesecloth, the filtrate was centrifuged at 2,500 rpm for 8 min. The extracted solution was made up to 100 mL with distilled water. The supernatant was used for analysis of ascorbic acid.

•Titration of okra pods:

1- 25 ml of okra juice was added to 125 ml Erlenmeyer flask.

2- Then it was titrated until the endpoint reached. (Iodine solution was added until got a blue-black color).

3- The titration was repeated until had at least three measurement that agree within 0.1 ml.

• Titration Calculations

- The ml of used titrant was calculated or each flask. The measurements were taken 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 the sample.

3.1.1.5.7 Measurement of potassium (K) content

The K content of pod was determined by using a Cardy Potassuim meter instantly after harvesting the pods. One gram of pod was homogenized in 5 ml distilled water in mixer and centrifuged at 4000 rpm for 10 min. Then 3 drops of the supernatant liquid were dropped onto the calibrated sensor pad (Cardy Potassium Meter, Model-2400, USA), on a sampling paper placed on the sensor. The reading in ppm was taken from the display pad after it stabilized (30 to 43 sec).

3.1.1.5.8 Nutrient contents

Analysis of nutritional contents of okra (Fe, Ca, Mg and Na) was done using Multi element analyzer (MEA). Samples were grounded properly using green pod by mortar and pestle. 5 ml water was mixed with the sample. After that 1ml of the sample was injected to the MEA and reading were calculated.

3.2 OVARY INJECTION METHOD APPLICATION OF PLANT GROWTH REGULATORS AT DIFFERENT CONCENTRATIONS.

3.2.1 Experiment 2

Effect of ovary injection method on okra growth and development by using Indole acetic acid (IAA)); Naphthalene acetic acid (NAA) and Gibberellic acid (GA_3)

3.2.1.1 Study site and Climatic information

The same site was selected for this experiment which was described in 3.1.1.

3.2.1.2 Plant materials

Plant materials were same as described in 3.1.1.

3.2.1.3 Application of IAA; NAA and GA_3

After blooming stage, 0.5 ml of the various concentrations of 0 and IAA**;** NAA and GA_3 (25, 50, 100 and 200 mg/l) were applied to the flower ovary with the needle for a surgical purpose (Plate 3.3) while control ovary was treated with distilled water mixed with 2ml of 1% ethanol. Four flowers were chosen randomly for each replication.

3.2.1.4 Data collection and analysis

Data were recorded considering following parameters:

3.2.1.4.1 Pod setting

Same as described in 3.1.1.5.1

3.2.1.4.2 Pod parameters

Same as described in 3.1.1.5.2

3.2.1.4.3 Single pod weight (Average)

Same as described in 3.1.1.5.3

3.2.1.4.4 Seed production

• Healthy seeds/pod (%)

• Aborted seeds/pod (%)

Same as described in 3.1.1.5.4

3.2.1.4.5 Total soluble solid (TSS)

Same as described in 3.1.1.5.5.

3.2.1.4.6 Measurement of Vitamin C

Same as described in 3.1.1.5.6.

3.2.1.4.7 Measurement of the K content

Same as described in 3.1.1.5.7.

3.2.1.4.8 Nutrient contents

Same as described in 3.1.1.5.8.

Plate 3.3. Photo shows injecting hormone solutions into the ovary.

3.3 STEM INJECTION METHOD APLLICATION OF PLANT GROWTH REGULATORS AT DIFFERENT CONCENTRATIONS.

3.3.1 Experiment 3

Effect of stem injection method on okra growth and development by using Indole acetic acid (IAA)); Naphthalene acetic acid (NAA) and Gibberellic acid (GA_3)

3.3.1.1 Study site and Climatic information

The same site was selected for this experiment which was described in 3.1.1.

3.3.1.2 Plant materials

Plant materials were same as described in 3.1.1.

3.3.1.3 Application of IAA; NAA and GA_3

One and an half ml (1.5ml) of the various concentrations of control and IAA; NAA and GA_3 (25, 50, 100 and 200 mg/l) were applied on the stem by injected the plant stems with needle for a surgical purpose of 1 dose at the height of 3 cm above the ground level (Plate 3.4). While control solution was distilled water mixed with 2ml of 1% ethanol.

Plate 3.4. Photo shows stem injection technique.

3.3.1.5 Measurement of parameters

Data were recorded considering the following parameters:

3.3.1.5.1 Plant height and stem girth (cm)

Plant height was measured from above ground level up to the uppermost tip of the leaves at the end of harvesting. Both plant height and stem girth were measured using a meter rule with the aid of thread.

3.3.1.5.2 Leaf numbers

Number of leaves: number of leaves on each treated and control plants were counted.

3.3.1.5.3 Leaf chlorophyll content

The chlorophyll content in the leaves was measured by SPAD value meter (Minolta Japan). The mean of three readings from a handy Minolta chlorophyll meter Spectrum, Japan) was achieved for each leaf disc from individual leaves (4 leaves per plant). The leaf disc used to obtain a SPAD value provided sufficient tissue for total chlorophyll.

3.3.1.5.4 Leaf chlorophyll fluorescence measurement

Fast chlorophyll fluorescence was evaluated on the upper surface of latest fully expanded leaf by using a Plant Efficiency Analyzer (PEA, Hansatech Instruments Ltd., England). A leaf clip was appended to the leaf and kept in the dark for 15 minutes for dark adaptation. After that the shutter plate was opened and light was applied on the leaf. The initial fluorescence intensity (Fo) when all reaction centers (RCs) are open, the maximal fluorescence intensity when all reaction are close (Fm), the variable fluorescence (Fv = Fm-Fo) and the time to reach the maximal fluorescence intensity (tmax), were calculated. The quantum yield was determined according to the equation Fv/Fm. Used the ratios Fv/Fm and which provide an estimation of the maximal photochemical efficiency of photosystem II (Ouzounidou *et al*., 2006) to evaluate alterations under our experimental conditions.

3.3.1.5.5 Number of branches

Number of branches was counted at the end of harvesting stage by physical counting.

3.3.1.5.6 Days to first flowering

First day of flowering was determined when the petals of the first flower were fully opened in the control and the treated plants.

3.3.1.5.7 Number of pods per plant

Mature pods were picked at harvest stage when they were tender at the edible portion.

3.3.1.5.8 Pod parameters

Same as described in 3.1.1.5.2

3.3.1.5.9 Single pod weight (Average)

Same as described in 3.1.1.5.3

3.3.1.5.10 Seed production

- Healthy seeds/pod (%)
- Aborted seeds/pod (%)

Same as described in 3.1.1.5.4.

3.4 SEED- PRETREATMENT METHOD

Pre-sowing treatments was applied to okra (*Abelmoschus esculentus*) seeds with Indole acetic acid (IAA); Naphthalene acetic acid (NAA) and Gibberellic acid (GA_3) at different concentrations.

3.4.1 Experiment 4

Soaked seed in (IAA, NAA and GA_3) and grow them in field (*In vivo*)

3.4.1.1 Study site and Climatic information

The same site was selected for this experiment which was described above in 3.1.1.

3.4.1.2 Application of IAA; NAA and GA_3

Hormone solutions of 0, 25, 50, 100 and 200 mg/l of IAA; NAA and GA_3 were prepared separately. The okra seeds for each concentration soaked in 10 ml of the treatment solution for 24 hours. They were occasionally shaken for freshening and in order to keep them at a uniform moisture level. After that the seeds redried to original weight with spreading them over the blotting paper for 48 hours at room temperature (~ 28 ºC) (Sundstrom ea al., 1987). These were planted directly in the field. The depth of planting was 1.5 cm from the surface of the soil. Three seeds were planted in each hole and later thinned down to one (Plate 3.5). While control solution was distilled water mixed only with 2 ml of 1% ethanol.

Plate 3.5. Photograph shows seed soak technique plant.

3.4.1.3 Data collection and analysis

Data was recorded considering following parameters:

3.4.1.3.1 Germination (%)

The appearance of the plumule at the soil surface was taken as germination. Germination records were taken everyday for two weeks. For the determination of germination percentage was calculated using this formula

$$\text{Germination (\%)} = \frac{\text{Total numbers of growing plumule}}{\text{Total number of planted seeds}} \times 100$$

3.4.1.3.2 Days of germination

Days of germination were determined by recorded the duration of the first seedling emergence on the ground surface.

3.4.1.3.3 Measurement of parameters

Data were recorded considering the following parameters:

3.4.1.3.4 Plant height and stem girth (cm)

Same as described in 3.3.1.5.1

3.4.1.3.5 Leaves number

Same as described in 3.3.1.5.2

3.4.1.3.6 Leaf chlorophyll content

Same as described in 3.3.1.5.3

3.4.1.3.7 Leaf chlorophyll fluorescence measurement

Same as described in 3.3.1.5.4

3.4.1.3.8 Number of branches

Same as described in 3.3.1.5.5

3.4.1.3.9 Days to first flowering

Same as described in 3.3.1.5.6

3.4.1.3.10 Number of pods per plant

Same as described in 3.3.1.5.7

3.4.1.3.11 Pod parameters

Same as described in 3.3.1.5.8

3.4.1.3.12 Seed production

• Healthy seeds/pod (%)\

• Aborted seeds/pod (%)

Same as described in 3.1.1.5.4.

3.4.1.3.13 Total soluble solid (TSS)

Same as described in 3.1.1.5.5.

3.4.1.3.14 Measurement of Vitamin C

Same as described in 3.1.1.5.5.

3.4.1.3.15 Measurement of the K content

Same as described in 3.1.1.5.6.

3.4.1.3.16 Nutrient contents

Same as described in 3.1.1.5.7.

3.5 GROW SOAKED SEEDS ON MURASHIGE AND SKOOG'S MEDIUM *(In vitro)*

3.5.1 Experiment 5

Indole acetic acid (IAA) Naphthalene acetic acid (NAA) and Gibberellic acid (GA_3)

3.5.1.1 Seed sterilization

Before inoculation on culture medium:

1- The healthy seeds were kept in gauze and washed with Teepol.

2- Kept in running water for 30 minutes.

3- They were washed with distilled water for 3-5 times

4- Surface sterilized by soaking them in the solution of 70% Clorox plus 3 drops of Tween 20 for 1minute then rinsed them in sterile dH2O one time.

5- Soaked them again in 50% Clorox for 1min after that washed them again with dH2O one time.

6- Soaked the seeds in 70% ethanol for 1 min and finally by rinsing three times with sterile water.

3.5.1.2 Media preparation

Seeds were cultured on MS (Murashige and Skoog, 1962) medium supplemented with 3% sucrose and solidified with 0.8 % agar. pH of the medium was adjusted to 5.8 before autoclaving.

1- 800 ml of distilled water was filled in the baker.
2- 4.4 g of MS powdered medium was added in the beaker.
3- 30 g of sucrose was added.
4- PH at (5.75- 5.8) was set.
5- 8 g agar technical (No.3) was added to the beaker
6- The media autoclaved at 121ºC for 21 minutes.

6- Then okra seeds soaked in 10 ml of three different plant growth regulators, IAA; NAA and GA_3 at 0, 25, 50,100 and 200 mg/l concentrations for 24 hours.

7- These seeds transferred to Culture media after soaking.

Seeds were germinated after five days of culture with normal roots, shoots and leaves (Plate 3.6).

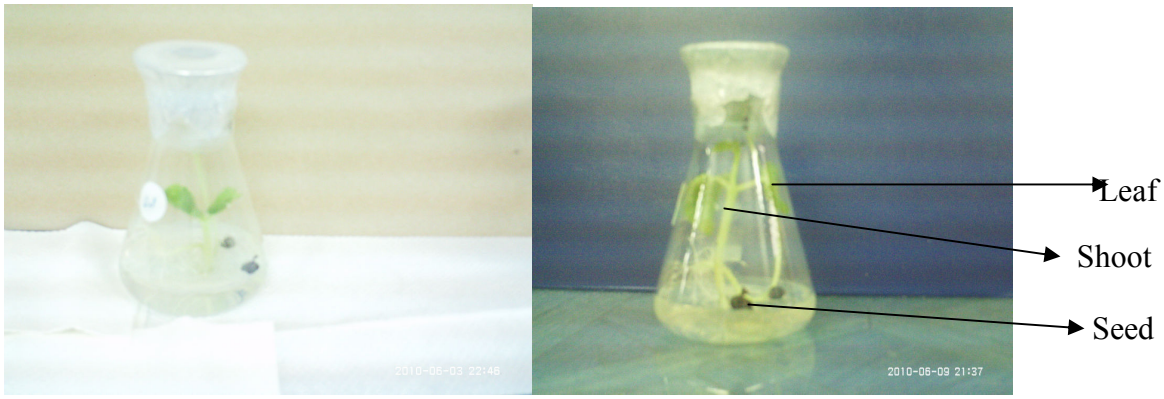

Plate 3.6. Photo shows germinated seeds in culture medium with normal roots, shoots and leaves.

3.5.1.3 Acclimatization

After germination occurred at 2 -3 leaf stages with good rooting system plants were transplanted to soil. All of shoots were washed, cleaned from agar and planted in polybags. All the polybags were filled with sandy loam soil. One plantlet was planted in each polybags. The polybags were saturated with water and enclosed by polyethylene and placed under shade house conditions. After 8 days, the polyethylene enclosure was partly opened and after 14 days was removed and plantlets were watered. After 25 days of polyethylene removal, all of the plantlets transferred to Banting field in order to grow into normal plants (Plate 3.7).

Plate 3.7. Photo shows the plantlets (*in vitro*) transferred to Banting field in order to grow in the normal environment.

3.5.1.4 Data collection and analysis

Data was recorded considering following parameters:

3.5.1.4.1 Germination (%)

Same as described in 3.4.1.4.1

3.5.1.4.2 Days of germination

Same as described in 3.4.1.4.2

3.5.1.4.3 Measurement of parameters

Data were recorded considering the following parameters:

3.5.1.4.4 Plant height and stem girth (cm)

Same as described in 3.3.1.5.1

3.5.1.4.5 Leaves number

Same as described in 3.3.1.5.2

3.5.1.4.6 Leaf chlorophyll content
Same as described in 3.3.1.5.3

3.5.1.4.7 Leaf chlorophyll fluorescence measurement
Same as described in 3.3.1.5.4

3.5.1.4.8 Number of branches
Same as described in 3.3.1.5.5

3.5.1.4.9 Days to first flowering
Same as described in 3.3.1.5.6

3.5.1.4.10 Number of pods per plant
Same as described in 3.3.1.5.7

3.5.1.4.11 Pod parameters
Same as described in 3.3.1.5.8

3.5.1.4.12 Seed production
• Healthy seeds/pod (%)\
• Aborted seeds/pod (%)
Same as described in 3.1.1.5.4.

3.6 EXPERIMENTAL DESIGN

The experiment was laid out in randomized block design having four replications. The whole area was divided into fifteen blocks and each block contains 20 unit plots. The size of the unit plot was 1 x 1 m^2.

3.7 STATISTICAL ANALYSIS

The obtained data were statistically analysed using SPSS Computer Programme, Version16. 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.

CHAPTER 4

RESULTS

4.1 FLOWER INJECTION METHOD APPLICATION OF PLANT GROWTH REGULATORS AT DIFFERENT CONCENTRATIONS

Experiment 1

Effect of Flower injection method on okra growth and development by using Indole acetic acid (IAA); Naphthalene acetic acid (NAA) and Gibberellic acid (GA_3)

4.1.1 Effect of different concentrations of IAA applied as flower injection method on okra growth and seed production.

4.1.1.1 Pod setting, growth, development and seed production

The percentage of pod setting per plant of okra was significantly influenced by flower injection application of different concentrations of IAA as shown in Figure 4.1. The concentrations of IAA (25, 50 mg/l) greatly increased the percentage of pod setting per plant compared to the control. Meanwhile, concentrations of 100, 200 mg/l IAA treatments decreased when compared with control. The percentage of pod set of okra was 78% in the control. Results for IAA flower injection treatment at concentration of 25 mg/l showed a higher percentage of pod set: 95-100 %, while IAA treatment at 200 mg/l produced the lowest percentage of pod setting: 55-60 % in comparison to the other treatments.

The effect of various concentrations of IAA on the mean pod length, pod diameter, pod size and weight of okra pod are shown in Table 4.1. IAA flower injection application with different concentrations had significant differences in pod length compared with the control. The maximum pod length (13.29 cm) was observed with 25 mg/l while minimum pod length

(4.28) was recorded in the control pod (Plate 4.1). Pod diameter was also significantly affected by different concentrations of IAA compared with control. The highest mean value of pod diameter was obtained for 25 mg/l IAA concentration (2.33). This value was significantly different ($\alpha = 0.05$) from those obtained for 0, 50, 100 and 200 mg/l IAA concentrations. Application of IAA increased the weight of pods. The maximum response in this parameter was reached by applying the lowest concentration (19.52±0.02) while the lowest response was with control (2.98±0.05).

The seed yield is presented in Table 4.2. In the present investigation, higher healthy seed percentage per plant (88.33%) was recorded at flower injection treatment with 25 mg/l concentration (88.33%) followed by control mg/l treatment and 50 mg/l, which recorded 84.30 % per plant and 57.80 % per plant, respectively. Application of higher dose of IAA (200 mg/l) registered zero percent of healthy seeds (Plate 4.2). Data presented in Table 4.2 showed that the highest concentration of IAA (200 mg/l) in this investigation inhibited seed production and produced 100% of Aborted seeds.

4.1.1.2 Total soluble solids (TSS), vitamin C (Vit. C) and nutrient content

Total soluble solids (TSS) were markedly increased by IAA application at different concentrations. Under IAA treatment okra pod presented the highest TSS was with 25 mg/l concentration while the lowest value was in control treatment. IAA at 100 and 200 mg/l showed similar results in total soluble value, but TSS value was high at 50 mg/l., On another hand, results shown in Table 4.1 illustrate that the vitamin C in okra, pods was significantly increased by IAA treatment. Vitamin C pod content was increased over the control at 25, 50 and 100 mg/l. IAA at 25 mg/l resulted in the highest pod content of vitamin C while

treatment with 200 mg/l IAA had the least effect on vitamin C content. In 50 and 100 mg/l, these treatments also resulted in greater vitamin C content compared to the control.

There are some important minerals highly concerned with human nutrition, such as calcium potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and sodium (Na) were analyzed in samples of okra pods. These minerals are very important for many metabolic and immune processes in the human body. In the present study, data in Table 4.3 showed that okra plants with IAA at all the used level led to obvious increase in the endogenous content of potassium (K). The highest value were obtained from the application of 25 mg/l (97.36 mg/100g) followed by 50, 100 and 200 mg/l (94.23 mg/100g), (93.06 mg/100 g) and (92.21 mg/100g), respectively. Meanwhile, the control produced the lowest content of potassium (91.81 mg/100g). The results also show that the calcium content in pods ranged from 50.73 to 58.24 mg/100g. IAA all concentrations decreased the calcium pod content, except 25 mg/l IAA. The control pods have the highest Ca contents (58.24 mg/100g). Furthermore, magnesium (Mg) and sodium (Na) content significantly were decreased by IAA application. Maximum content of both minerals was achieved from control pods (39.25 and 6.22 mg/100), respectively. IAA application at (25 mg/l) produced no significantly different effect ($P>0.05$) relative to control. On the contrary, iron (Fe) content increased significantly by the application of IAA with whole concentrations compared to control. The maximum content of Fe content was 0.484 mg/100 g with the lowest concentration in this study (25 mg/l).

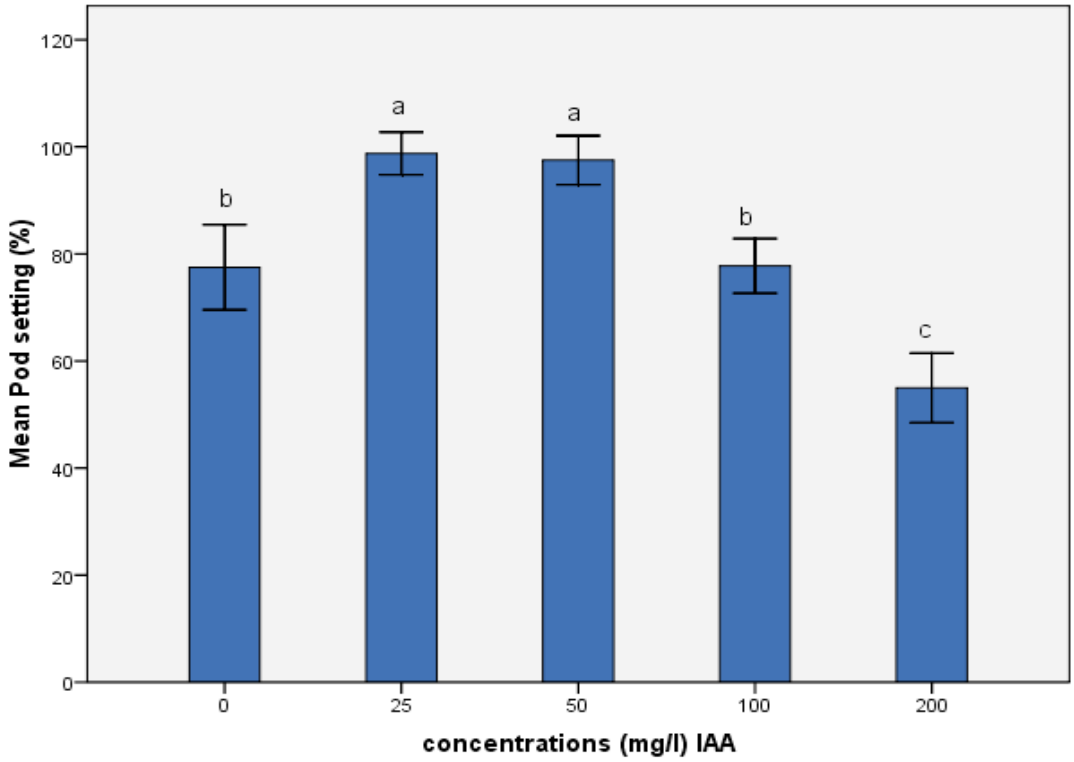

Figure 4.1. Pod setting percentage in okra was influenced by different concentration indole acetic acid (IAA) with flower injection technique.

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.1. Effect of various concentrations of IAA on physical characters and nutrient composition of okra pods by flower injection method.

Concentrations (mg/l)	Pod length (cm)	Pod diameter (cm)	Pod size (cm^2)	Single pod weight /treated flower(g)	T.Soluble solid/pod (%Brix)	Vit.C/pod (mg/100g)
0	4.28±0.02e	1.77±0.03e	7.60±0.12e	2.98±0.05e	2.22±0.03d	11.75±0.01c
25	13.29±0.02a	2.42±0.03a	32.16±0.37a	19.52±0.02a	2.77±0.05a	18.86±0.03a
50	11.50±0.01b	2.33±0.02b	26.79±0.26b	16.33±0.03b	2.55±0.02b	12.80±0.01b
100	11.10±0.01c	2.01±0.02c	22.31±0.31c	13.33±0.05c	2.34±0.08c	12.78±0.02b
200	7.39±0.02d	1.92±0.03d	14.20±0.24d	8.98±0.07d	2.34±0.02c	11.77±0.02c

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

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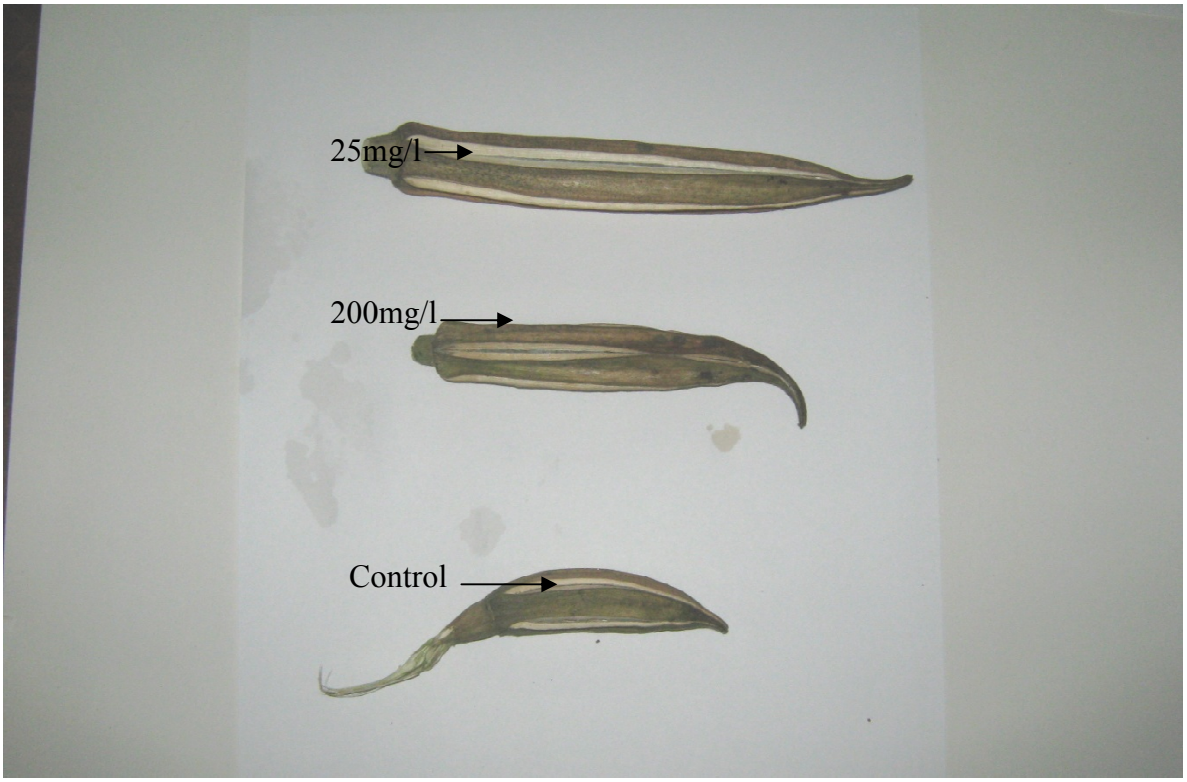

Plate 4.1. Photograph shows the pod harvested from flower injection treatment with IAA at 25, 200 and 0 mg/l.

Table 4.2. Effect of different levels (mg/l) IAA on seed yield of okra pods by flower injection method.

Concentrations (mg/l)	Healthy seeds/plant (%)	Aborted seeds/plant (%)
0	84.30±0.02b	15.70±0.02d
25	88.33±0.03a	11.70±0.03e
50	57.80±0.03c	42.21±0.03c
100	32.20±0.02d	67.90±0.02b
200	0.00e	100a

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).
0: No seeds

Plate 4.2. Photograph shows influence of IAA on the injected flower treatment on aborted seed percentage of okra at different concentrations.

Table 4.3 Mineral elements (mg/100g) contents of okra were affected by different concentrations (mg/l) of IAA.

Concentrations mg/l	K (mg/100g)	Ca (mg/100g)	Mg (mg/100g)	Fe (mg/100g)	Na (mg/100g)
0	91.81±0.03e	58.24±0.01a	39.25±0.02a	0.421±0.1e	6.22±0.01a
25	97.36±0.01a	57.20±0.01a	39.25±0.13a	0.484±0.2a	6.20±0.02ab
50	94.23±0.03b	55.67±0.02b	36.19±0.13b	0.479±0.1b	6.18±0.02b
100	93.06±0.02c	53.63±0.014c	33.65±0.06c	0.463±0.1c	6.07±0.01c
200	92.21±0.03d	50.73±0.04d	32.15±0.01d	0.446±0.1d	5.94±0.01d

Values are mean ± standard deviation.
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

4.1.2 Effect of Flower injection method on okra growth and development by using Naphthalene acetic acid (NAA)

4.1.2.1 Pod setting, growth, development and seed production

The flower injection application of naphthalene acetic acid had significant effects on increasing pod setting per plant with 25 mg/l concentration compared with control (95.5 and 77.5 %), respectively. Moreover, NAA treatments with the concentrations 50 and 100 mg/l had a lower pod setting percentage compared to 25 and 0 mg/l. But treatment with NAA at 200 mg/l concentration inhibited the pod setting (0%). Yield contributing characters of okra were significantly influenced by different concentrations of NAA as shown in Table 4.4. Pod length was significantly increased with 25 (8.97 cm), respectively (Plate 4.3). Significantly highest pod diameter was registered with 25 followed by 50 mg/l. The single pod weight per plant increased following 25 and 50 mg/l, respectively. On another hand, 100 mg/l treatment had the least effect on pod characters compared to other concentrations of NAA but still higher than control. In addition, these results showed that healthy seed percent reflected higher value in NAA 25 and 50 mg/l treated flowers (94.35, 88.68%), respectively over control (84.30%) whereas NAA 100 mg/l treated flowers exhibited lower healthy seeds' percent (51.22%) than control (Figure 4.2). Percentage of aborted seeds was highest in NAA 100 mg/l (48.51%), pods of control followed next (15.69%). NAA at 25 mg/l treatment had the lowest percentage of aborted seeds (5.63%) compared to other treatments (Figure 4.3).

4.1.2.2 Total soluble solids (TSS), ascorbic acid and nutrient content

Data showed that flower injecting okra flowers with naphthalene acetic acid (NAA) caused the significant effects on TSS and vitamin C pod content (Table 4.5). Generally, all the NAA concentrations (25 and 50 and 100 mg/l) caused significant increase in TSS pod content

compared with control. Maximum content of TSS (2.83% Brixº) per pod was achieved by 25 and 50 mg/l of NAA applied to flowers. Moreover, both concentrations of NAA (25 and 50 mg/l) increased significantly vitamin C content per pod. Maximum vitamin C content (14.98 mg/100g) was recorded in the pod harvested from 25 mg/l NAA treated flowers, and found in descending order: 50 mg/l (12.80 mg/100g), control (11.75 mg/100g) and 100 mg/l (11.36 mg/100g), respectively. Likewise, potassium (K) content per pod was also found significantly increased among concentration treatments. Minimum mean potassium content in the pod was recorded in pods with 100 mg/l and control (92.22 and 92.21 mg/100g), respectively. Whereas NAA application did not show any significant effect ($P>0.05$) on calcium content per pod at rate 25, 50 mg/l compared to control, the higher concentration (100 mg/l) decreased it compared to other treatments.

In The results also show that 50 and 100 mg/l decreased the magnesium (Mg) and sodium (Na) contents per pod except 25 mg/l did not show any effect on both contents per pod compared to control (Table 4.6).

Table 4.4. Pod setting (%), length, diameter, size and weight under flower injection method with NAA at different concentrations (mg/l) in okra plants.

Concentrations (mg/l)	Pod setting (%)	Pod length (cm)	Pod diameter (cm)	Pod size (cm^2)	Single pod weight (g)
0	77.50±0.50b	4.28±0.02d	1.77±0.03d	7.60±0.12d	2.98±0.05d
25	97.50±0.50a	8.97±0.03a	2.40±0.02a	21.52±0.29a	13.41±0.03a
50	65.00±5.77c	6.30±0.02b	2.22±0.03b	13.98±0.41b	9.11±0.03b
100	45.00±5.77d	5.20±0.02c	1.84±0.02c	9.56±0.09c	4.10±0.02c
200	0	0	0	0	0

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).
0: No setting

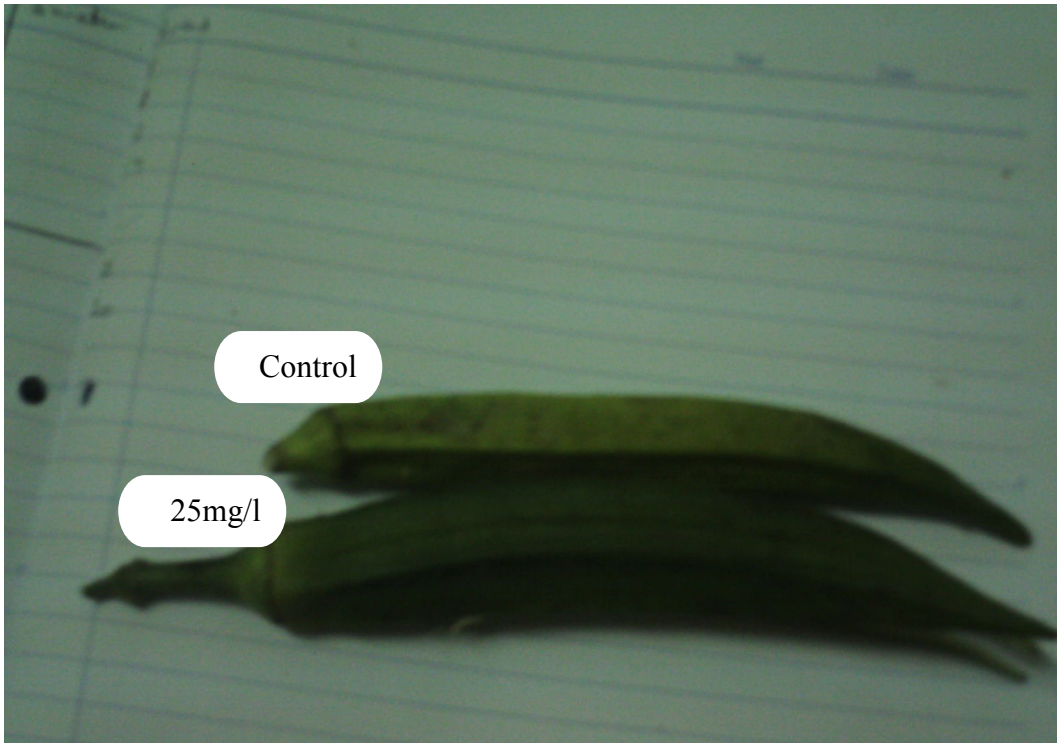

Plate 4.3. Photograph shows okra pods harvested from control flower (0 mg/l) and treated flower with 25 mg/l concentrations of NAA.

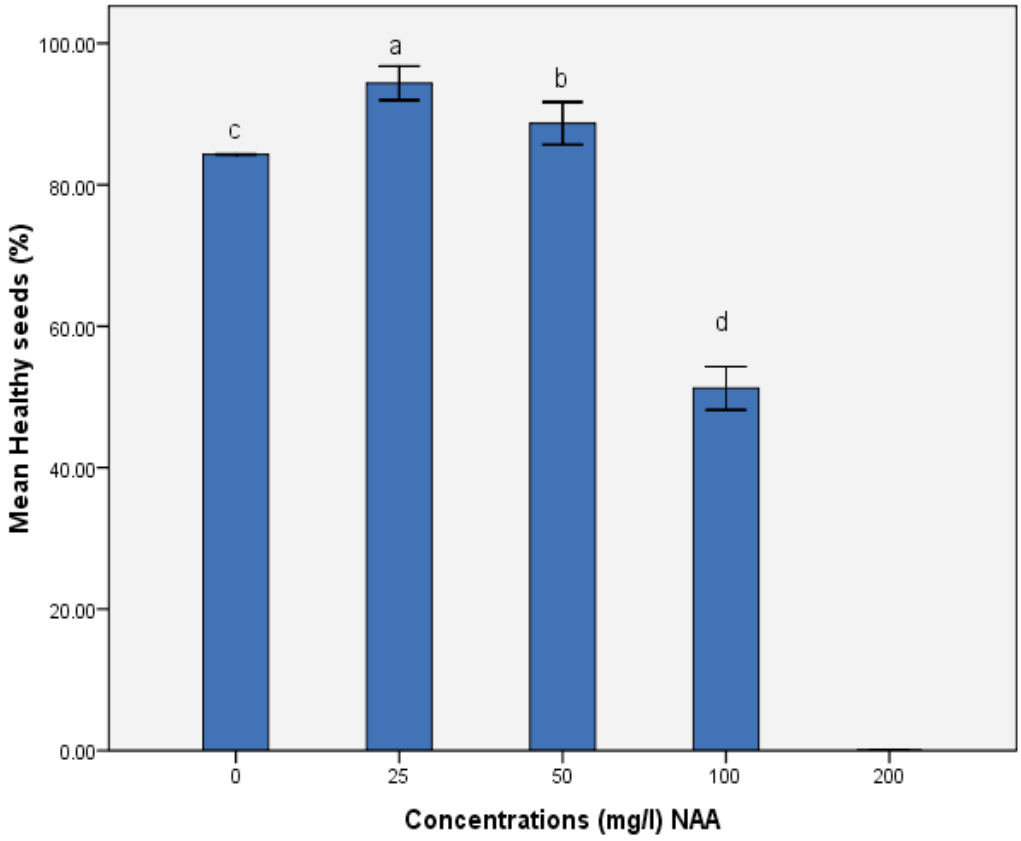

Figure 4.2. Healthy seed percentage (%) was affected by concentrations (mg/l) of NAA flower injection application in okra.

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

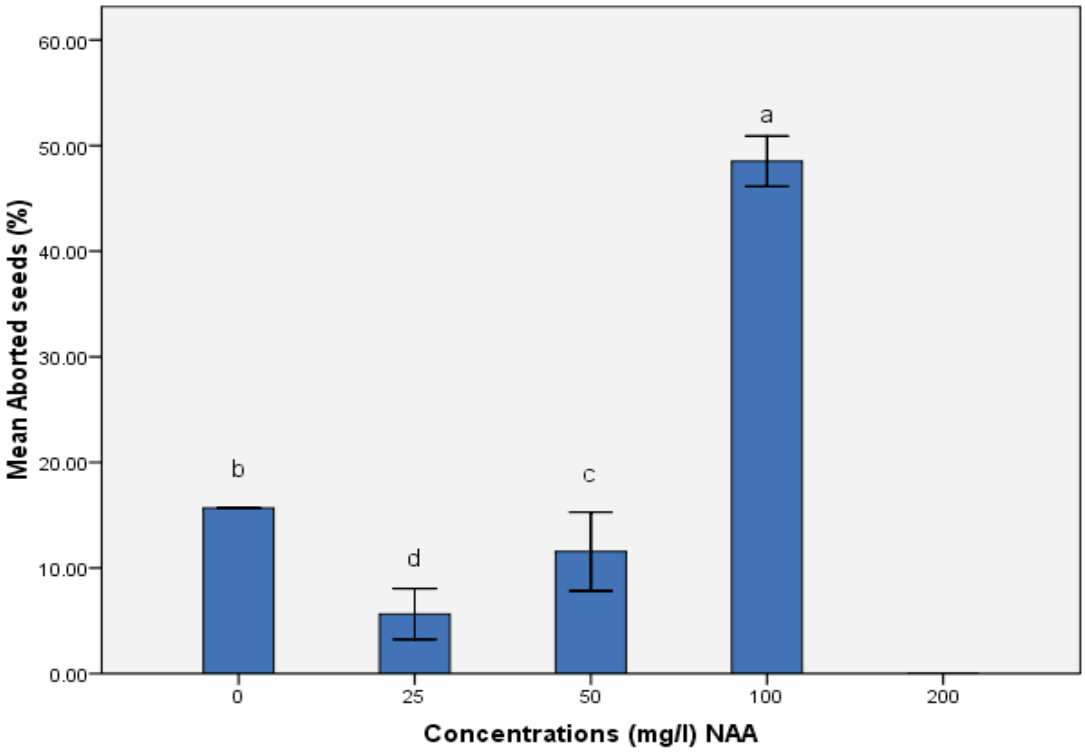

Figure 4.3. The effect of different concentrations (mg/l) of NAA on aborted seed percentage (%).

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.5. Effect of NAA on total soluble solid, vitamin C, potassium and calcium of okra pod at different concentrations (mg/l) applied by flower injection.

Concentrations (mg/l)	T. Soluble solid/pod (%Brix)	Vit. C/pod (mg/100g)	K/pod (mg/100g)	Ca/pod (mg/100g)
0	2.22±0.02c	11.75±0.013c	92.21±0.03c	58.25±0.01a
25	2.83±0.022a	14.98±0.02a	95.98±0.024a	58.24±0.01a
50	2.82±0.024a	12.80±0.06b	93.66±0.10b	58.23±0.02a
100	2.55±0.037b	11.36±0.02d	92.22±0.02c	57.08±0.02b
200	0	0	0	0

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT)
0: No setting

Table 4.6. Effect of different concentrations (mg/l) NAA on nutritional content of okra pods.

Concentrations (mg/l)	Mg (mg/100g)	Fe (mg/100g)	Na (mg/100g)
0	39.26±0.02a	0.421±0.01d	6.22±0.01a
25	39.26±0.02a	0.460±0.01a	6.22±0.01a
50	36.15±0.01b	0.454±0.01b	6.05±0.02b
100	33.60±0.01c	0.434±0.02c	5.93±0.01c
200	0	0	0

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT)
0: No setting

4.1.3 Effect of Flower injection method on okra growth and development by using Gibberellic acid (GA_3)

4.1.3.1 Pod setting and growth development

The results (Figure 4.4) indicated that GA_3 had significantly increased pod setting as compared to control. Maximum pod setting after flower injection (100%) was obtained in case of GA_3 at 100 mg/l, which significantly differed from control and was at par with 50 mg/l (98.75%). However, 25 and 200 mg/l treatments also significantly differed from control (95 and 88.25%), respectively.

The data (Table 4.7) further showed that maximum pod length (10.59 and 10.58 cm) was observed by applying GA_3 of 100 and 50 mg/l while minimum length (4.28 cm) was obtained in control (0 mg/l). GA_3 at 25 and 200 mg/l increased the pod length over control (6.90 and 8.30 cm), respectively. Statistically, all concentrations of GA_3 gave significantly higher length of pod than control. In addition, the results revealed that pod diameter was also affected with GA_3 application. Highest diameter pod (2.29 cm) was recorded in application of 50 mg/l GA_3 followed by 100, 200 and 25 mg/ (2.27, 2.20 and 2.19 cm). The control treatment produced minimum diameter (1.77cm). Pod diameter tended to increase with increasing GA_3 concentration up to 100 mg/l whereas it decreased at 200 but was higher than control. In this respect, plate 4.4 showed the pods harvested from control plants (0 mg/l applied), and plants treated with GA_3. It was evident that GA_3 application at all concentrations caused an increase in the size of the pods when compared with that harvested for control (0 mg/l). Pods were harvested from plants treated with GA_3 at all concentrations studied. At 50 and 100 mg/l GA_3 applications, the pod size was the highest when compared with that harvested for other treatments. In this respect, GA_3 had stimulatory effects on

individual pod weight per plant. The data revealed that GA_3 at 50 and 100 mg/l produced the heaviest pods (10.75 g both) followed by 200 and 25 mg/l (8.42 and 7.42 g), respectively. Control treatment produced the lightest pods (2.98 g). Effect of GA_3 on percentage of healthy and aborted seeds was recorded (Table 4.8).

4.1.3.2 Seed production; total soluble solids (TSS) and vitamin V (Vit. C)

All the treatments had been increasing trend towards production of healthy seeds compared with control. Treatments with GA_3 at rates 50, 100 and 200 mg/l had maximum healthy seeds (95%) per pod; It statistically was higher than 25 mg/l and control (93.43% and 84.30% respectively). Minimum healthy seeds (84.30%) were observed in control treatment. Significant differences among treatments were observed in seed abortion intensity, which was maximum with control treatment (15.70 %) in followed by 25 mg/l (6.56%), while the minimum (4.30%) in 200 mg/l.

The results also showed that GA_3 flower injection at all concentrations influenced the biochemical parameters of okra pods (Table 4.8). The statistical analysis of data showed the significant effect of GA_3 on TSS of pod content. Maximum TSS (3.89% Brixº) was observed in 100 mg/l treatment, which differed significantly from all other treatments. It was followed by 50 mg/l (3.24 % Brixº). GA_3 concentrations up to 200 mg/l increased TSS content of pods. Like other nutrient constituents, vitamin C content was also appreciably affected with GA_3 application, which ranged from 11.75 to 15.90 mg/100g of pod being the minimum in control and maximum in 100 mg/l.

4.1.3.3 Nutrient content

The results presented in Table 4.9 revealed that potassium pod content was also affected with GA_3 flower injection application at different concentrations. Maximum content (99.14 mg/100g) was recorded in application of 100 mg/l followed by 50 mg/l (95.93 mg/100g), 25 mg/l (93.86 mg/100g) and 200 mg/l (94.19 mg/100g). Control treatment produced the lowest pod content of potassium (92.21mg/100g).

In the contrary, flower injection application with GA_3 had significantly reduced pod contents of Ca, Mg and Fe as compared to control. Maximum pod contents of Ca, Mg and Fe (58.24, 39.26 and 0.42 mg/100g, respectively) were recorded in control followed by 25 mg/l (58.09, 39.19 and 0.417 mg/100g, respectively) and 50 mg/l (57.03, 39.02 and 0.411 mg/100g, respectively). However, 100 and 200 mg/l treatments also significantly differed from control. In another word, GA_3 at concentration 100 mg/l was reduced pod content of Ca, Mg and Fe (56.74, 38.87 and 0.408 mg/100g, respectively) and 200 mg/l (56.16, 38.32 and 0.403 mg/100), respectively. However, the analysis of variance showed that flower injection with GA_3 at various concentrations exerted highly varied influence on sodium (Na) pod content. The highest Na content per pod (7.06 mg/100g) was obtained by 100 mg/l treatment followed by 200 mg/l (6.92 mg/100g) and the lowest content (6.21 mg/100g) was observed with control treatment. The data indicated that the higher concentrations of GA_3 increased Na pod content efficiently than the lower concentrations.

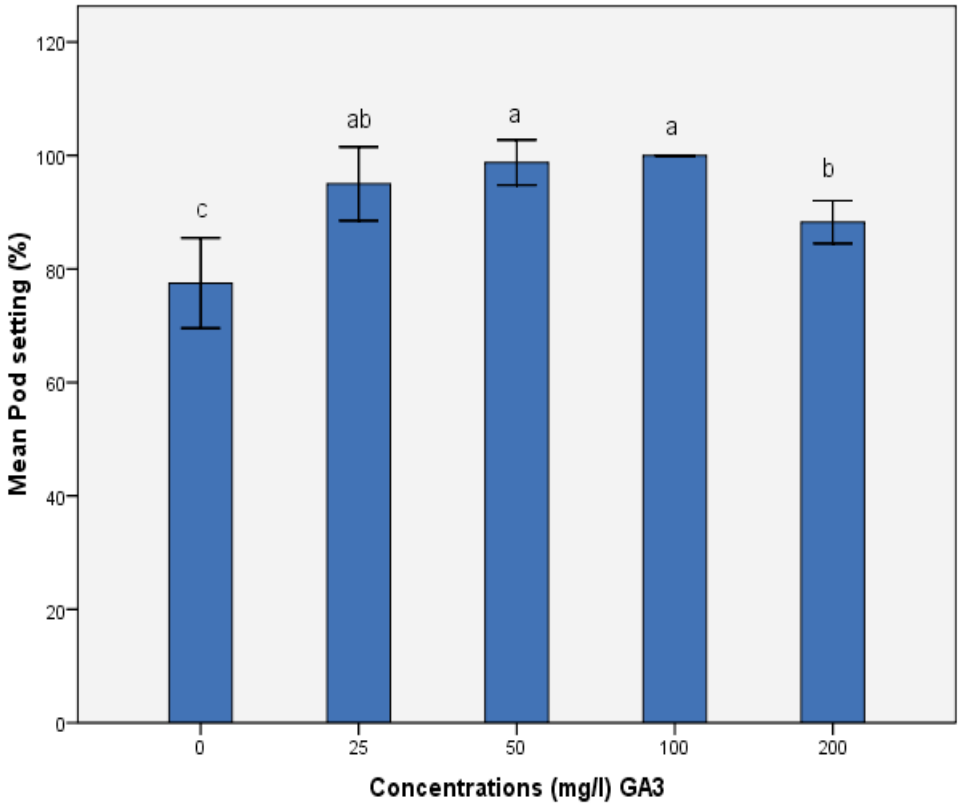

Figure 4.4. The effect of different concentrations (mg/l) of GA_3 on pod setting percentage of okra plant applied by flower injection method.

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.7. Effect of different concentrations (mg/l) GA_3 on growth characters of okra plant.

Concentrations (mg/l)	Pod length (cm)	Pod diameter (cm)	Pod size (cm^2)	Single pod weight (g)
0	4.28±0.02d	1.77±0.03c	7.60±0.12d	2.98±0.05d
25	6.90±0.02c	2.19±0.02b	15.11±0.26c	7.42 ±0.02c
50	10.58±0.02a	2.29±0.02a	24.22±0.39a	10.75±0.03a
100	10.59 ±0.05a	2.27±0.04a	24.03±0.63a	10.75±0.02a
200	8.30±0.03b	2.20±0.01b	18.26±0.27b	8.42±0.02b

Values are mean ± standard deviation

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMR) .

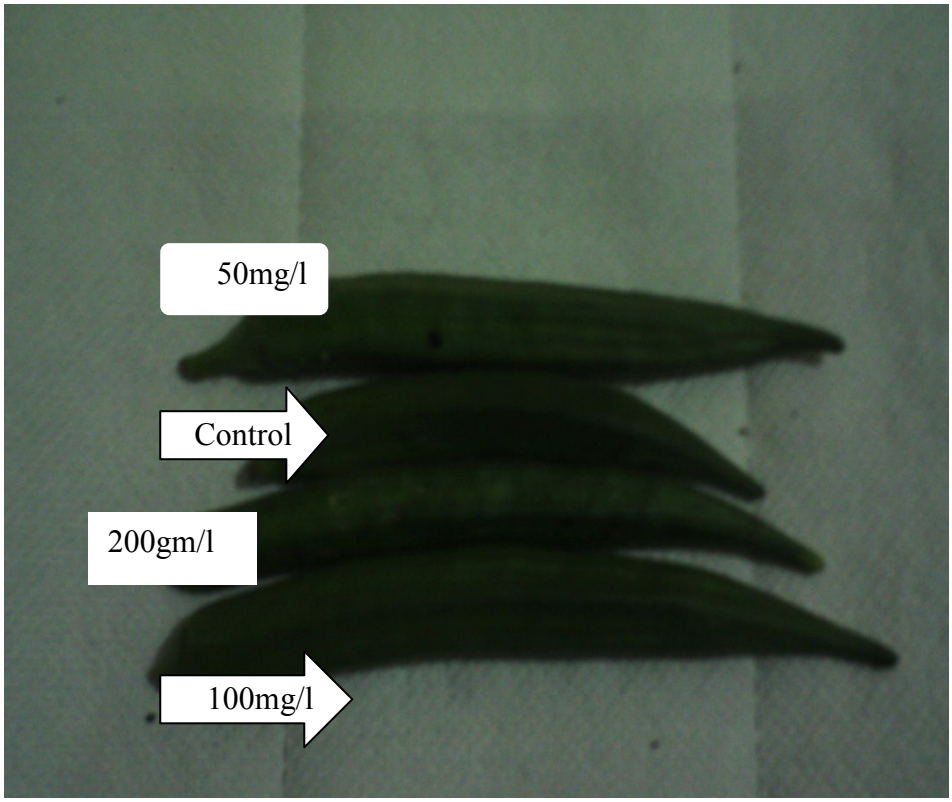

Plate 4.4. Photograph shows the pods harvested from control flowers and flowers treated with GA_3 at rates 50, 200 and 100mg/l concentrations.

Table 4.8. Healthy seeds (%), aborted seeds (%), total soluble solid and vitamin C of okra was affected by GA_3 flower injection application at different concentrations.

Concentrations (mg/l)	Healthy seeds (%)	Aborted seeds (%)	TSS (%Brix)	Vit. C (mg/100g)
0	84.30±0.02d	15.70±0.02a	2.22±0.03e	11.75±0.01e
25	93.43±0.04c	6.56±0.02b	2.96±0.04c	13.45±0.02d
50	95.19±0.03b	4.80±0.04c	3.24±0.02b	14.22±0.02b
100	95.68±0.01a	4.31±0.03d	3.89±0.02a	15.90±0.03a
200	95.69±0.03a	4.30±0.03d	2.65±0.02d	13.90±0.02bc

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT)

Table 4.9. Nutritional elements (mg/100 g) of okra pods were affected by different concentrations of GA_3 using flower injection method.

Concentrations mg/l	K (mg/100g)	Ca (mg/100g)	Mg (mg/100g)	Fe (mg/100g)	Na (mg/100g)
0	92.21±0.03e	58.25±0.01a	39.26±0.02a	0.421±0.01a	6.22±0.01e
25	93.86±0.02c	58.09±0.10b	39.19±0.02b	0.417±0.02a	6.78±0.01d
50	95.93±0.05b	57.03±0.02c	39.02±0.01c	0.411±0.01b	6.86±0.01c
100	99.14±0.03a	56.74±0.12d	38.87±0.01d	0.408±0.03b	7.06±0.02a
200	94.19±0.02d	56.16±0.02e	38.32±0.02e	0.403±0.01c	6.93±0.02b

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

4.2 APPLICATION OF PLANT GROWTH REGULATORS AT DIFFERENT VIA OVARY INJECTION METHOD

Experiment 2

Effect of ovary injection method on okra growth and development by using Indole acetic acid (IAA); Naphthalene acetic acid (NAA) and Gibberellic acid (GA_3)

4.2.1 Effect of different concentrations of IAA applied as ovary injection method on okra growth and seed production.

4.2.1.1 Pod setting and growth development

A comparison of the data in Table 4.10 indicated significant variations for all pod characters studied. Highest pod setting percentage was recorded at 25 mg/l (99.77%) and the lowest in the 100 mg/l (28.70%) as compared to control (71.46). Similarly, the highest pod length was observed with 25 mg/l (10.15cm) followed by 50 mg/l treatment (5.92cm) and control (3.19 cm). Furthermore, IAA application at 25 and 50 mg/l led to increase the pod diameter by 6-20%, respectively. Similar results were obtained in pod size and pod weight where 25 and 50 mg/l ovary treatment induced an increase in both parameters which they are related together. However, there was a marked decrease in the pod characters with the increase in concentration and these were lower at higher concentration (100 mg/l) than control. However pod setting percentage of the ovary treated with 200 mg/l was so poor that only very few pods set in the plants. As a result of this, measurements of this treatment were not made.

4.2.1.2 Seed production; total soluble solids (TSS) and vitamin C

Results presented in table 4.11 showed that the percentage of healthy seeds reduced with the increase of IAA concentrations. The highest percentage of healthy seeds was exhibited by

control treatment (72.15%) while the IAA at 100 mg/l level showed inhibited effect of seed growth (Plate 4.5).

The statistical of data showed the significant effect of IAA on pod content of TSS. Maximum TSS (2.85% Brixº) was obtained in 25 mg/l treatment, which differed significantly from all treatments. It was followed by 50 mg/l (2.44% Brixº) as compared to control (2.30% Brixº). IAA concentration up to 100 mg/l decreased TSS content of pods (2.21% Brixº). IAA at lower concentrations (25 mg/l and 50 mg/l) produced significantly more vitamin C content of pods (15.93 and 11.63 mg/100 g) than control (10.74 mg/100 g).

4.2.1.3 Nutrient content

It was observable from table (4.12) that the pod content of K, Mg, Fe and Na were significantly affected by IAA treatments at different concentrations. The IAA-100 mg/l gave the lowest pods content of K, Mg and Na (91.12, 38.83 and 2.82 mg/ 100g) as compared to control treatment (91.53, 39.18 and 6.15 mg/100 g), respectively. Highest contents of K and Fe was noted for IAA-25 mg/l (94.16 and 0.472 mg/l), followed by IAA-50 mg/l (92.32 and 0.45 mg/100 g). In particular, all concentrations of IAA showed decreased pods content with Mg per pod in comparison with control (39.18 mg/100 g). Likewise, IAA application at all concentrations produced no significantly different effects ($p > 0.05$) relative to control, on pods content with calcium in test okra samples.

Table 4.10. Effect of IAA on the pod yield of okra applied by ovary injection.

Concentrations (mg/l)	Pod setting (%)	Pod length (cm)	Pod diameter (cm)	Pod size (cm^2)	Single pod weight (g)
0	71.46±0.04b	3.19±0.02c	1.66±0.02c	5.31±0.10c	2.76±0.03c
25	99.77±0.14a	10.15±0.02a	2.07 ±0.03a	21.09±0.28a	15.56±0.02a
50	68.70±0.01c	5.92±0.02b	1.76±0.02b	10.46±0.10b	6.12±0.02b
100	28.70±0.11d	3.13±0.03d	1.39±0.02d	4.36±0.09d	2.22±0.02d
200	0	0	0	0	0

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).
0: No germination

Table 4.11. Effect of IAA injected okra ovary on seed production and biochmical characters.

Concentrations (mg/l)	Healthy seeds/pod (%)	Aborted seeds/pod (%)	Soluble solid/pod (%Brix)	Vit. C /pod (mg/100 g)
0	72.15±0.03a	27.84±0.03d	2.30±0.02c	10.74±0.01d
25	46.24±0.02b	53.74±0.02c	2.85±0.01a	15.93±0.02a
50	24.12±0.02c	75.86±0.02b	2.44±0.02b	11.63±0.02b
100	0d	100.00±0.00a	2.21±0.02d	10.85±0.03c
200	0	0	0	0

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).
0: No germination

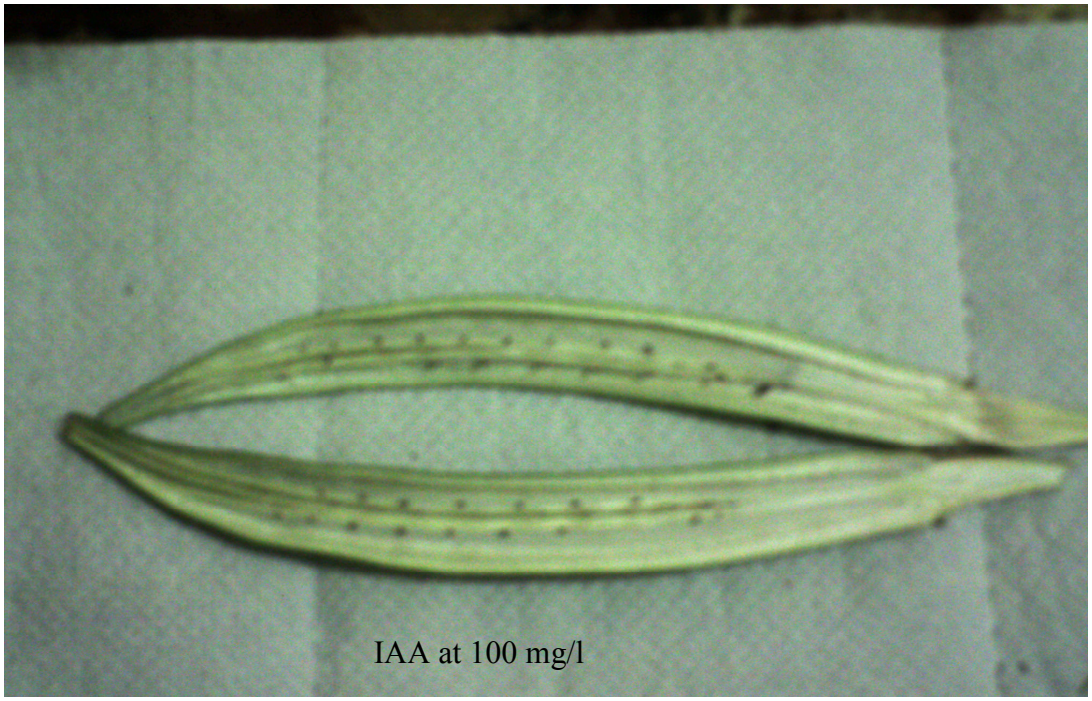

Plate 4.5. Photograph shows the effect of IAA on seed production by ovary injection method.

Table 4.12. Effect of different concentrations of IAA on mineral elements (mg/100 g) of okra pod applied by ovary injection.

Concentrations mg/l	K	Ca	Mg	Fe	Na
0	91.53±0.03c	56.15±0.02a	39.18±0.02a	0.409±0.001c	6.15±0.03a
25	94.16±0.02a	56.15±0.01a	39.10±0.02b	0.472±0.002a	6.07±0.11a
50	92.32±0.02b	56.12±0.01a	39.04±0.02c	0.454±0.002b	5.91±0.02c
100	91.12±0.04d	56.12±0.48a	38.83±0.03d	0.412±0.001c	2.82±0.02d
200	0	0	0	0	0

Values are mean ± standard deviation
Duncan Multiple Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Range Test (DMRT).
0: No germination

4.2.2 Effect of ovary injection method on okra growth and development by using Naphthalene acetic acid (NAA)

4.2.1.1 Pod setting growth, development, total soluble solids (TSS) and vitamin C (Vit.C)

The percentage of pod set was 71% in the control (Figure 4.5). Results for NAA ovary treatment at lower concentration (25 mg/l) showed a higher percentage of pod set: 29% over the control. IAA application at 50 mg/l was 95% while 100 mg/l ovary treatment was decreased the percentage of pod setting (28%). Otherwise, no pod set occurred in NAA ovary treatment at 200 mg/l.

On another hand, Data presented in Table (4.13) showed that NAA ovary treatment at various concentrations significantly influenced the average of pods characters and pod content of TSS and vitamin C when compared with the control pods. NAA at 25 and 50 mg/l ovary treatments significantly increased the average of pod length (10.25 and 5.21 cm) compared to control pods (3.19 cm) but NAA at 100 mg/l treatment was decreased pod length by 15%. Results also indicated that the synthetic growth regulator NAA at lower concentrations (25 and 50 mg/l) had the better effect on pod diameter, size and individual weight than control. On another hand, NAA treatment at 200 mg/l was reduced pod diameter, pod size and pod weight by 26, 37 and 30%, respectively. Results were shown in the same table demonstrate that the total soluble solids (TSS) and vitamin C in pods were higher at 25 mg/l treatment (2.77 and 15.78 mg/100 g) which indicated that doses produced better quality pods as compared to control and other concentrations.

4.2.1.2 Seed production

The data on percentage of healthy and aborted seeds are presented in figure 4.6. In the present investigation, highest healthy seed percentage per pod (84.2%) was recorded at 25 mg/l level of NAA while the highest aborted seed percentage was observed at 100 mg/l. Higher concentrations of NAA (50, 100 mg/l) decreased the percentage of healthy seeds (42.3 and 30.2%) and increased aborted seed percentage (57.6 and 69.8%) as compared to control (72.8 and 27.8%), respectively (Plate 4.6).

4.2.1.3 Nutrient content

The effect of NAA at different concentrations applied by ovary injection method on content with K, Ca, Mg, Fe and Na per pod was shown Table 4.14. Although pods content of potassium and iron increased with NAA applications at 25 and 50 mg/l (93.93 and 92.30 mg/100 g) Ca, Mg and Na decreased with 25 mg/l application (54.09, 38.43 and 6.11 mg/100 g) as compared to control. However, all of these contents were decreased by application of 100 mg/l when compared to control.

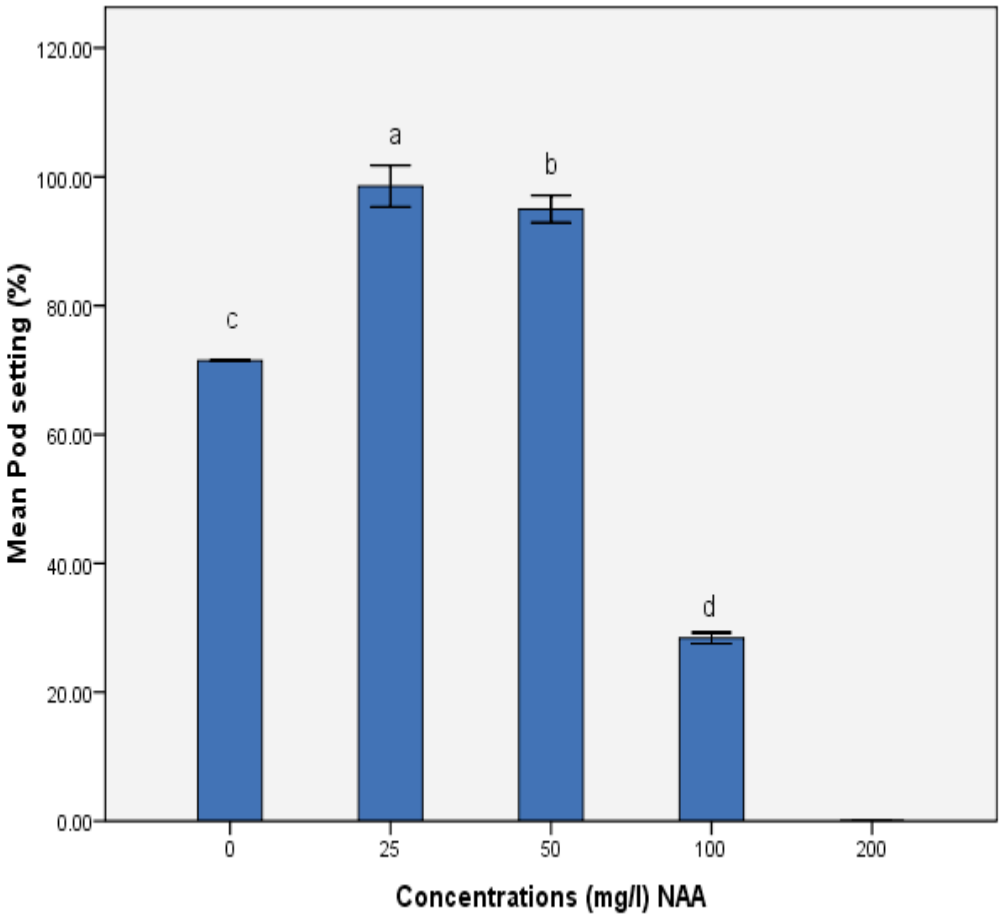

Figure 4.5. Effect of different concentrations of NAA on pod setting (%) of okra pod was applied by ovary injection.

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.13. Effect of NAA application on physical characters and nutrient composition of okra pod applied by ovary injection method.

Concentrations (mg/l)	Pod length (cm)	Pod diam. (cm)	Pod size (cm^2)	Single pod weight (g)	TSS/pod (%Brix)	Vit.C/pod (mg/100 g)
0	3.19±0.02c	1.66±0.02c	5.31±0.10c	2.76±0.03c	2.30±0.020b	10.74±0.01c
25	10.25±0.02a	2.22±0.024a	22.53±0.26a	10.20±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	2.29±0.011b	11.32±0.02b
100	2.72±0.03d	1.23±0.03d	3.37±0.11d	1.92±0.02d	2.13±0.02c	10.40±0.03d
200	0	0	0	0	0	0

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).
0: No germination

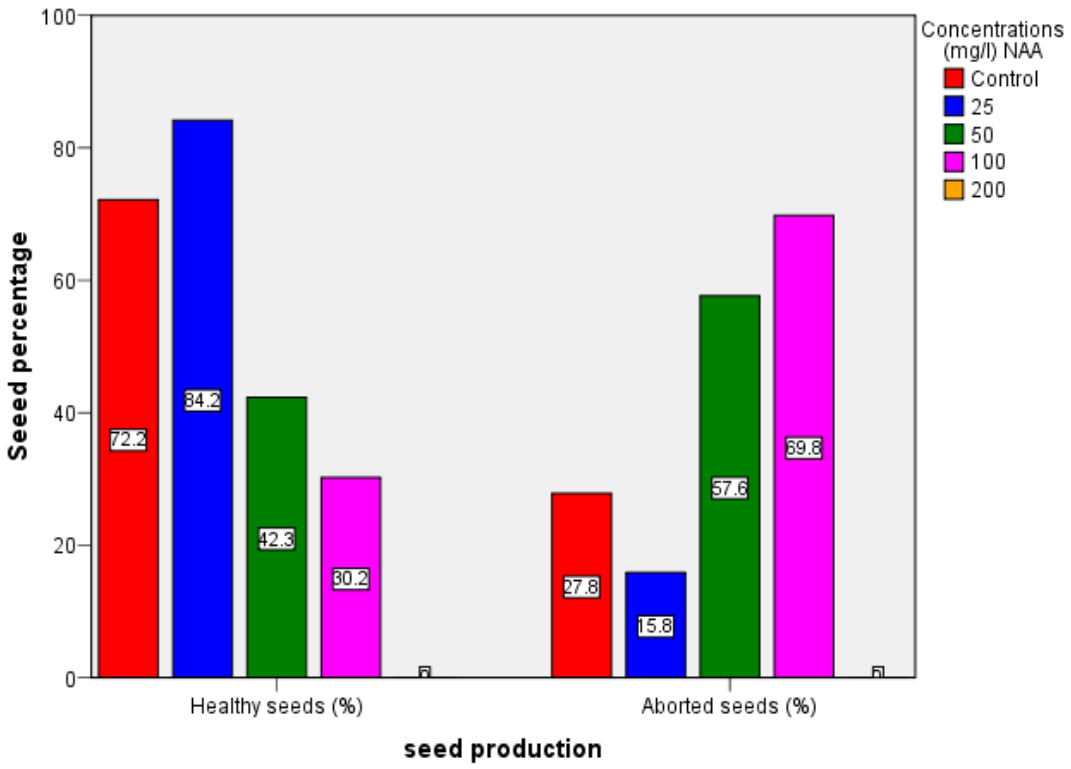

Figure 4.6. Seed production percentage of okra plants influenced by NAA at different concentrations applied by ovary injection.

Plate 4.6. Influence of NAA at 100 mg/l on seed production of okra applied by ovary injection method.

Table 4.14. Effect of different concentrations of NAA on mineral elements (mg/100 g) of okra pod applied by ovary injection.

Concentrations mg/l	K	Ca	Mg	Fe	Na
0	91.53±0.03c	56.15±0.02a	39.18±0.02a	0.409±0.001c	6.15±0.03a
25	93.93±0.05a	54.09±0.02b	38.43±0.02b	0.445±0.002a	6.11±0.01a
50	92.30±0.02b	51.92±0.02c	37.60±0.02c	0.424±0.001b	4.80±0.01b
100	90.49±0.02c	50.40±0.02d	36.45±0.01d	0.408±0.002c	2.61±0.03c
200	0	0	0	0	0

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).
0: No germination

2.3. Effect of ovary injection method on okra growth and development by using Gibberellic acid (GA_3)

4.2.3.1 Pod setting, growth and development

The results (Figure 4.7) indicated that GA_3 had the significantly the effect on pod setting as compared to control. Maximum pod setting (99.50 and 99.42%) occurred in GA_3 25 and 50 mg/l ovary treatment whereas minimum pod setting (71%) observed in case of NAA at 200 mg/l and control. In addition, 100 mg/l ovary treatment significantly differed from control where it enhanced pod setting 20% over control.

As regards to pod characters, the control GA_3 (25 and 50 mg/l) produced bigger pod size with heavier pod weight than other levels. The biggest and heaviest (24.73 cm and 18.16 g) pod was with 50 mg/l followed by 25 mg/l (23.35 cm and 17.34 g) whereas the smallest and lightest pods were produced by control treatment (5.31 cm and 2.76 g). This differed significantly at ($P<0.05$) (Table 4.15). The results further indicate that GA_3 applications with higher concentrations (100 and 200 mg/l) significantly decreased all pod characters, but they were higher than control.

4.2.3.2 Seed production, total soluble solids (TSS) and vitamin C (Vit. C)

The ovary injection of GA_3 had the significant effect on healthy and aborted seed percentage per pod compared with control (Table 4.16). The percentage of healthy seeds was highest in GA_3 50 mg/l ovary treatment (95.15%), healthy seed percentage of GA_3 100 mg/l followed next (92.38%). Application of GA3 with 200 mg/l had lower healthy seed percentage (70.80%) compared to other treatments. Significant differences among treatments were observed in seed abortion intensity, which was the maximum (29.20%) in 200 mg/l

treatment followed by control (27.84%), while the minimum (4.85%) in 50 mg/l. Results also indicated that the ovaries that were injected with GA_3 at concentrations of 25 and 50 mg/l had highest pod content of TSS (2.94 and 3.15% Brixº) if compared with control (2.30% Brixº). It observed from table 4.16, the ovary injection application of GA_3 all concentrations increased the pod content of vitamin C. Like other nutrient ingredients; vitamin C content per pod was moreover, significantly affected with GA_3 application, which ranged from 10.74 to 15.57 mg/100 g of pod being the minimum in control and maximum in 50 mg/l.

4.2.3.3 Nutrient content

Effect of GA_3 application at various concentrations on nutrient content of pods (K, Ca, Mg, Fe and Na) was shown in Table 4.17. Potassium content of pods of treated ovaries varied from 91.70 to 98.63 mg/100 g and was generally higher in comparison with control (91.53 mg/100g), but the increase seemed not to be concentration dependent. The results also showed that the pod contents of calcium and iron significantly reduced them as a whole compared with control (56.15 and 0.409 mg/100 g). In addition, all the treatments had been decreasing trend towards magnesium (Mg) with control, except 25 mg/l treatment, which did not affect the pod content of Mg (39.19 mg/100 g) per pod; it was statistically same to the control (39.18). GA_3 treatment had significant difference in pod content of sodium (Na) compared with control. The maximum pod content (7 mg/100 g) was obtained with application of 50 mg/l and found in descending order: 25 mg/l (6.99 mg/l), 100 mg/l (6.43mg/100 g), 200 mg/l (6.21 mg/100 g) and control (6.15 mg/l) respectively.

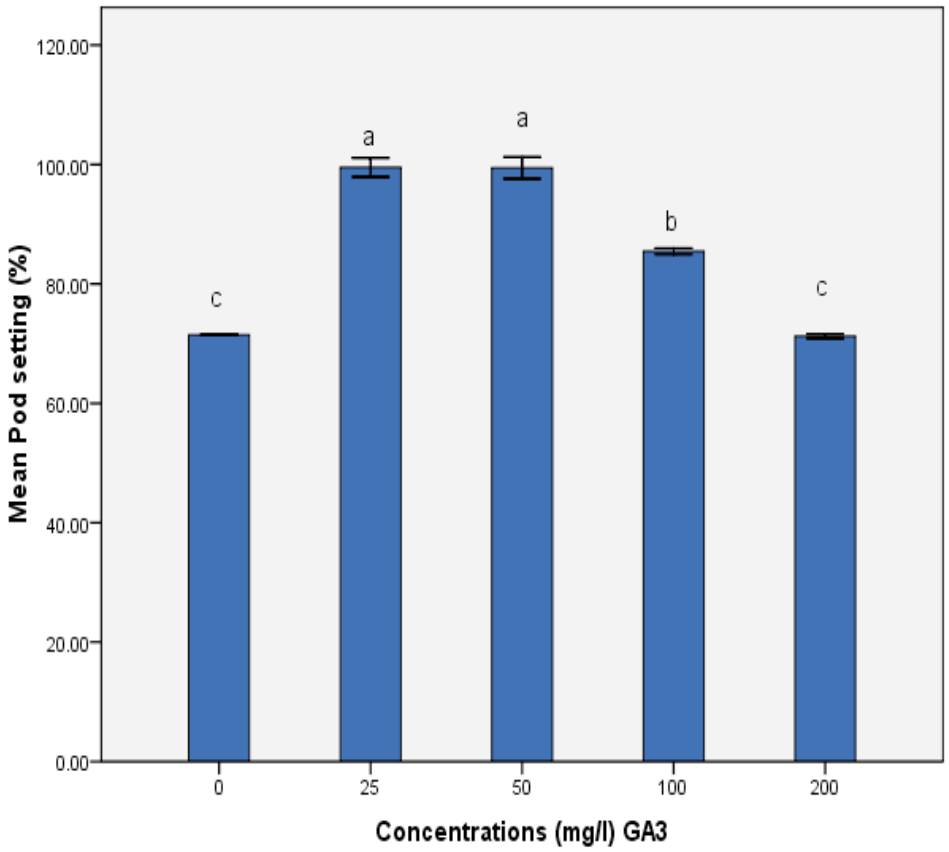

Figure 4.7. Pod setting percent (%) in okra as affected by GA_3 following ovary injection method.

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.15. Pod physical characters of okra under ovary injection method by GA_3 at various concentrations.

Concentrations (mg/l)	Pod length (cm)	Pod diameter (cm)	Pod size (cm^2)	Single pod weight (g)
0	3.19±0.02e	1.66±0.02e	5.31±0.10e	2.76±0.03e
25	10.74±0.02b	2.17 ±0.02b	23.35±0.16b	17.34±0.02b
50	11.03±0.03a	2.24±0.02a	24.73±0.24a	18.16±0.02a
100	7.35±0.02c	1.94±0.02c	14.28±0.11c	11.22±0.03c
200	7.20±0.0d	1.80±0.02d	12.96±0.13d	10.42±0.02d

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.16. Effect of ovary injection treatment with GA_3 on seeds yields, soluble solid (%Brix) and vitamin C (mg/100 g) of okra.

Concentrations (mg/l)	Healthy seeds/pod (%)	Aborted seeds/pod (%)	TSS/pod (%Brix)	Vit. C/pod (mg/100 g)
0	72.15±0.03d	27.84±0.03b	2.30±0.02d	10.74±0.01e
25	86.71±0.01c	13.29±0.01c	2.94±0.02b	15.33±0.02b
50	95.15±0.02a	4.85±0.02e	3.15±0.02a	15.57±0.02a
100	92.38±0.02b	7.62±0.02d	2.42±0.02c	13.31±0.03c
200	70.80±0.02e	29.20±0.03a	2.42±0.02c	10.90±0.01d

Values are mean ± standard deviation
Mean followed by same letter was not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.17. Effect of different concentrations of GA_3 on mineral (mg/100 g) contents of okra pod applied by ovary injection.

Concentrations mg/l	K	Ca	Mg	Fe	Na
0	91.53±0.03e	56.15±0.02a	39.18±0.02a	0.409±0.02a	6.15±0.03d
25	97.47±0.02b	55.06±0.03b	39.19±0.02a	0.409±0.01a	6.99±0.03a
50	98.63±0.02a	54.22±0.03c	38.42±0.02b	0.407±0.04a	7.00±0.02a
100	93.57±0.02c	52.61±0.02d	36.88±0.02c	0.385±0.01b	6.43±0.02b
200	91.70±0.02d	50.21±0.02e	33.42±0.02d	0.385±0.02b	6.21±0.02c

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

4.3 STEM INJECTION METHOD APPLICATION OF PLANT GROWTH REGULATORS AT DIFFERENT CONCENTRATIONS

Experiment 3

Effect of Stem injection method on okra growth and development by using Indole acetic acid (IAA); Naphthalene acetic acid (NAA) and Gibberellic acid (GA_3)

4.3.1 Effect of different concentrations of IAA applied as stem injection method on okra growth and seed production.

4.3.1.1 Plant height, number of branches, stem girth and number of leaves

Data presented in table (4.18) clearly indicated that plant height, number of branches, stem girth and number of leaves per plant were significantly affected by different concentrations of IAA applied by stem injection method. The data revealed that IAA at 100 and 200 mg/l produced the tallest plants (79.96 and 83.31 cm) followed by 25, 50 mg/l and control treatments (78.84 and 78.83 cm) respectively. Application of IAA also had stimulatory effects on the number of branches per plant. IAA at 25 and 50 mg/l had the significantly higher branch number (3 and 3.50 respectively) over control (1.50). However, 200 mg/l applications had the highest branch number than other treatments (5.75). IAA applications at all concentrations (25, 50, 100 and 200 mg/l) were more efficient in stem elongation than control (2.74, 4.22, 4.73, 5.21 and 2.20 cm) respectively. In another hand, all concentrations of IAA application produced significantly difference effects ($p > 0.05$) on number leaves per plant in treated plants. Number of leaves/plant increased with an increase in concentrations of IAA with values ranged from 20 to 34 for control and 200 mg/l respectively.

4.3.1.2 Chlorophyll content, Fv/Fm yield and pods number

Data in table (4.19) showed that chlorophyll content per pod did not increase with an increase of IAA concentrations with value ranged from 40.43 to 40.44 Spad value for control and 200

mg/l respectively. There was no statistical difference found due to treatments. Similarly, Fv/Fm yield was not affected by the different application levels of IAA with values ranged from 0.691 to 0.692 for control and 200 mg/l respectively. Pod yields per plant increased with an increase of IAA concentrations ranged from 9.50 to 15.50 for control to 200 mg/l respectively. There were no statistical differences found between 25 and 50 mg/l treatments (10) but still higher than control (9.50) whilst the highest number of pods/plant observed with 200 mg/l application (15.50).

4.3.1.3 Pod contributing characters and seeds production per Pod

Effect of different concentrations of IAA on pod contributing characters and percentage of healthy and aborted seeds was also recorded (Table 4.20 and Table 4.21). The results showed that pod length and pod diameter did not increase with an increase of IAA concentrations. There was no statistical difference found due to treatments. Furthermore, pod size was not affected by all different applications of IAA with values ranged from 5.20 to 5.25 for control and 200 mg/l, respectively. Pod weight did not significantly differ from each other under IAA treatments. Likewise, healthy and aborted seed percentages did not influence by the different concentrations of IAA applied.

Table 4.18. Effect of stem injection with IAA at different concentrations on vegetative growth parameters of okra.

Concentrations (mg/l)	Plant height (cm)	No. of branches/plant	Stem girth (cm)	No. of leaves/plant
0	78.83±0.01c	1.50±0.50d	2.20±0.02e	20.00±1.41d
25	78.84±0.01c	3.00±0.00c	2.74±0.02d	24.00 ±1.71c
50	78.84±0.02c	3.50±0.57bc	4.22±0.03c	28.00±1.26bc
100	79.96±0.02b	4.50±0.57b	4.73±0.02b	30.50±1.29b
200	83.31±0.02a	5.75±0.50a	5.21±0.02a	34.00±1.25a

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.19. Effect of IAA at various concentrations applied by stem injection method on chlorophyll contents, Fv/Fm yield and number of pods of okra.

Concentrations (mg/l)	Chlorophyll content (Spad value)	Fv/Fm yield	No. of pods/plant
0	40.43±0.50	0.691±0.002	9.50±1.29c
25	40.43±0.01	0.691 ±0.001	10.00 ±1.41bc
50	40.44±0.02	0.692±0.002	10.00±1.41bc
100	40.45±0.01	0.693±0.002	11.75±0.95b
200	40.44±0.02	0.692±1.41	15.50±1.29a
	NS	NS	*

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).
*: Significant level at 0.05 levels
N .S. No significant difference

Table 4.20. Yield and yield contributing characters of okra as influenced by IAA at different concentrations applied by stem injection technique.

Concentrations (mg/l)	Pod length (cm)	Pod diameter (cm)	Pod size (cm^2)	Single pod weight (g)
0	4.23±0.02	1.23±0.01	5.20±0.08	2.72±0.02
25	4.23±0.01	1.23 ±0.02	5.21±0.06	2.73±0.02
50	4.24±0.01	1.23±0.01	5.21±0.05	2.73±0.01
100	4.25±0.01	1.24±0.02	5.27 ±0.07	2.74±0.01
200	4.24±0.01	1.23±0.03	5.25±0.05	2.74±0.01
	NS	NS	NS	NS

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).
N .S.: No significant difference.

Table 4.21. Effect of different concentrations of IAA on okra seed production (%).

Concentrations (mg/l)	Healthy seeds(%)	Aborted seeds/pod (%)
0	95.33±0.02	4.64±0.01
25	95.34±0.01	4.65 ±0.01
50	95.35±0.01	4.66±0.02
100	95.35±0.01	4.66±0.02
200	95.35±0.02	4.66±0.02
	NS	NS

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).
*: Significant level at 0.05 levels
N .S.: No significant difference.

4.3.2 Effect of Stem injection method on okra growth and development by using Naphthalene acetic acid (NAA)

4.3.2.1 Plant height, number of branches, stem girth and number of leaves

Effect of NAA at different concentrations applied by a stem injection method on plant height, number of branches per plant, stem from girth and number of leaves per plant were recorded (Table 4.22). The tallest plants were observed with lower concentration treatment (25 mg/l) with a mean value of 81.92 cm compared to other treatments while other treatments did not significantly differ from each other. The data revealed that, 25 mg/l of NAA produced the maximum number of branches (2.50) as compared to control (1.50). With stem girth, the resulted showed that stem girth significantly increased with an increase in the level of NAA applied to stem but 200 mg/l treatment was lower (2.16 cm). Numbers of leaves were not statistically different from one another where it ranged from 22 to 21.25 for control to 200 mg/l.

4.3.2.2 Chlorophyll content, Fv/Fm yield and pods number

The results also showed that chlorophyll content per leaf did not influence by the different concentrations of NAA applied (Table 4.23). This was beside found with the maximum quantum yield of primary photochemistry (Fv/Fm) where it ranged from (0.691 to 0.689) for control and 200 mg/l respectively. There were no statistical differences observed on the number of pods with whole concentrations.

4.3.2.3 Pod contributing characters and seeds production per pod

In the present study, NAA application at different concentrations produced no significantly different effects ($p > 0.05$) relative to control, on pod length, pod diameter, pod size, individual pod weight and the percentage of healthy and aborted seeds in test okra samples

(Table 4.24). There was no significant difference in the pod length and pod diameter produced from plant treated with NAA at various concentrations. Furthermore, there was no difference between concentrations of NAA in pod size and individual pod weight as compared to control. The results showed that the effect of NAA at different concentrations injected to stem was not affected on healthy and aborted seed percentages. However, NAA had no effect on pod contributing and seed production by stem injection method.

Table 4.22. Effect of stem injection method with NAA at different concentrations on vegetative growth parameters of okra.

Concentrations (mg/l)	Plant height (cm)	No. of branches/plant	Stem girth (cm)	No. of leaves/plant
0	78.83±0.01b	1.50±0.50ab	2.20±0.02cd	20.00±1.41
25	81.92±0.05a	2.50±0.57a	2.73±0.02a	23.25 ±1.50
50	78.85±0.01b	2.00±0.00ab	2.29±0.03b	22.75±1.26
100	78.86 ±0.02b	1.75±0.50ab	2.24±0.01c	22.25±2.21
200	78.85±0.01b	1.25±0.50b	2.16±0.02d	21.25±0.96
	*	*	*	NS

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).
*: Significant level at 0.05 levels
N .S: no significant difference.

Table 4.23. Effect of NAA with stem injection method at various concentrations on chlorophyll contents, Fv/Fm yield and pods number of okra.

Concentrations (mg/l)	Chlorophyll content (SPAD value)	Fv/Fm yield	No. of pods/plant
0	40.43±0.50	0.691±0.002	9.50±1.29
25	40.45±0.03	0.693 ±0.02	10.75 ±1.50
50	40.43±0.02	0.692±0.02	10.50±1.29
100	40.43±0.02	0.691±0.02	10.00±0.82
200	40.42±0.04	0.689±0.03	9.00±0.82
	NS	NS	NS

Values are means ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).
N .S: no significant difference.

Table 4.24. Yield and yield contributing characters of okra as influenced by NAA at different concentrations applied by stem injection technique.

Concentrations (mg/l)	Pod length (cm)	Pod diameter (cm)	Pod size (cm^2)	Single pod weight (g)	Healthy seeds/pod (%)	Aborted seeds /pod (%)
0	4.23±0.02	1.23±0.01	5.20±0.08	2.72±0.02	95.33±0.02	4.64±0.01
25	4.24±0.02	1.24 ±0.02	5.27±0.09	2.73±0.02	95.34±0.02	4.65 ±0.02
50	4.24±0.02	1.24±0.02	5.27±0.05	2.73±0.02	95.34±0.05	4.65±0.02
100	4.23±0.02	1.23±0.02	5.21±0.10	2.73±0.03	95.33±0.02	4.66±0.03
200	4.23±0.02	1.22±0.03	5.18±0.10	2.72±0.02	95.32±0.02	4.66±0.02
	NS	NS	NS	NS	NS	NS

Values are means ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).
N .S: no significant difference.

4.3.3 Effect of Stem injection method on okra growth and development by using Gibberellic acid (GA_3)

4.3.3.1 Plant height (cm), number of branches, stem girth, number of leaves and Chlorophyll content

Plant height was influenced by the application of GA_3 (Figure 4.8). GA_3 applied at different concentrations influenced the plant height significantly ($P<0.05$). The higher concentrations (100 and 200 mg/l) of GA_3 greatly increased the plant height (106.60 and 105.02 cm) compared to the control (78.95). The lower concentrations had lesser plant height (88.42 and 101.63 cm) but higher than the control.

A significant variation was evident in the number of branches per plant, stem girth, number of leaves and leave area due to the application of gibberellic acid (GA_3) at different concentrations (Table 4.25). The treated plants generated higher number of branches over control. Among the GA_3 application, 100 mg/l of GA_3 induced maximum number of branches (6.00) followed by 200 mg/l (4.75) and 50 mg/l (3.00) as compared to control (1.50). In the contrary, GA_3 application at 100 and 200 mg/l induced the highest value of stem girth (4.64 and 3.54 cm) over the control (2.20 cm). The analysis of variance showed that GA_3 application exerted highly varied influence on the leaf number per plant. The highest number of leaves per plant (80) was obtained by 100 mg/l followed by 200 mg/l (64), and the lowest leaf number/plant (22) was observed with control treatment. The data indicated that the higher concentration of GA_3 increased number of leaves more efficiently than the lower concentrations. Results in Figure (4.9) indicated that leaf content of chlorophyll was affected significantly by different concentrations of GA_3. The results showed that all concentrations of GA_3 (25, 50, 100 and 200 mg/l) increased chlorophyll content per leaf by 37, 45, 60 and 55% of the control.

4.3.3.2 Pod production, Yield contributing characters and seeds yield percentage per pod

Results in table (4.26) indicated that total pods numbers per plant, pod length; pod diameter, pod size, single pod weight and aborted seed percentage per pod were significantly affected by different concentrations of GA_3. Among the concentrations, 100 mg/l had the maximum number of pod per plant (41) followed by 200 mg/l (37.50) and 50 mg/l (31.50) in comparison with control (9.50). The data revealed that 100 mg/l produced the longest pod (11.28 cm) followed by 200 mg/l (10.12 cm). Pod diameter was found the maximum with 100 mg/l (3.67 cm) and 200 mg/l (3.33 cm) and followed by 50 mg/l (3.18 cm) and 25 mg/l (2.33 cm). Significantly highest pod size was obtained in 100 mg/l (41.39 cm^2) followed by 200 mg/l (33.69 cm^2). In this respect, pod weight recorded significantly highest in 100 mg/l (10.10 g). Second heaviest pods were obtained in 200 mg/l (9.32 g), 50 mg/l (8.74 g) and 25 mg/l (5.25 g).

Pod harvested from 25 mg/l treated plants had significant highest aborted seed percentage (9.32 %) and it was followed by 50 mg/l (6.81%), control (4.64%), 200 mg/l (3.59%) and 100 mg/l (2.18%). 100 and 200 mg/l treatments had been increasing the production of healthy seed compared with control (Figure 4.10). 100 mg/l treatment which had the maximum healthy seeds (97.81%) per pod followed by 200 mg/l (96.41%) while minimum healthy seed was observed in 25 mg/l treatment (90.67%). However, 100 mg/l application produced the longer and heavier pods (Plate 4.7) than control.

4.3.3.3 Flowering time

Table 4.27 showed the effect of IAA, NAA and GA_3 on flowering. IAA and NAA did not affect the date of flowering of okra plants while treated plants with GA_3 showed various effects on flowering time dependent on its concentration. However, 100 mg/l GA_3 proved to be most effective causing seventeen days early flowering in comparison to control. The high concentration of 200 mg/l GA3 also showed second early flowering (fourteen days earlier than control).

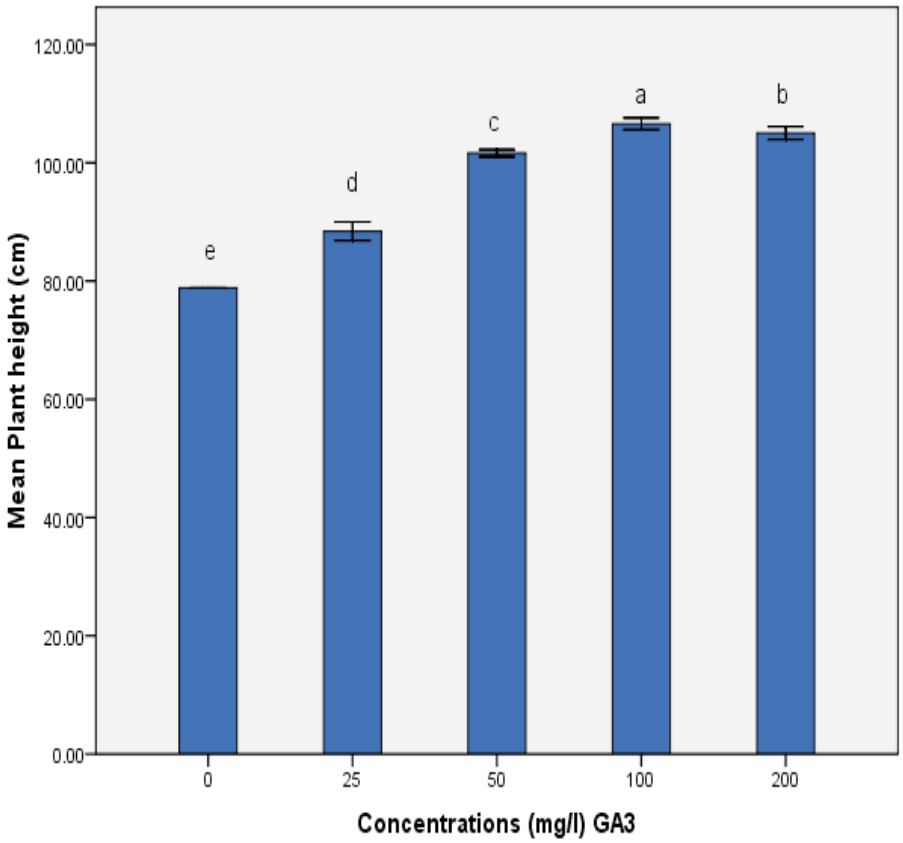

Figure 4.8. Effect of different concentrations of GA_3 on plant height of okra.

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.25. Effect of stem injection method applied with various concentrations of GA_3 on growth parameters of okra.

Concentrations (mg/l)	No. of branches/plant	Stem girth (cm)	No. of leaves/plant
0	1.50±0.50d	2.20±0.02d	20.00±1.41e
25	2.50±0.57c	2.20±0.01d	46.75±2.06d
50	3.00±0.01c	3.00±0.01c	52.50±1.29c
100	6.00 ±0.02a	4.64±0.01a	80.00±1.41a
200	4.75±0.50b	3.54±0.03b	64.00±0.82b

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

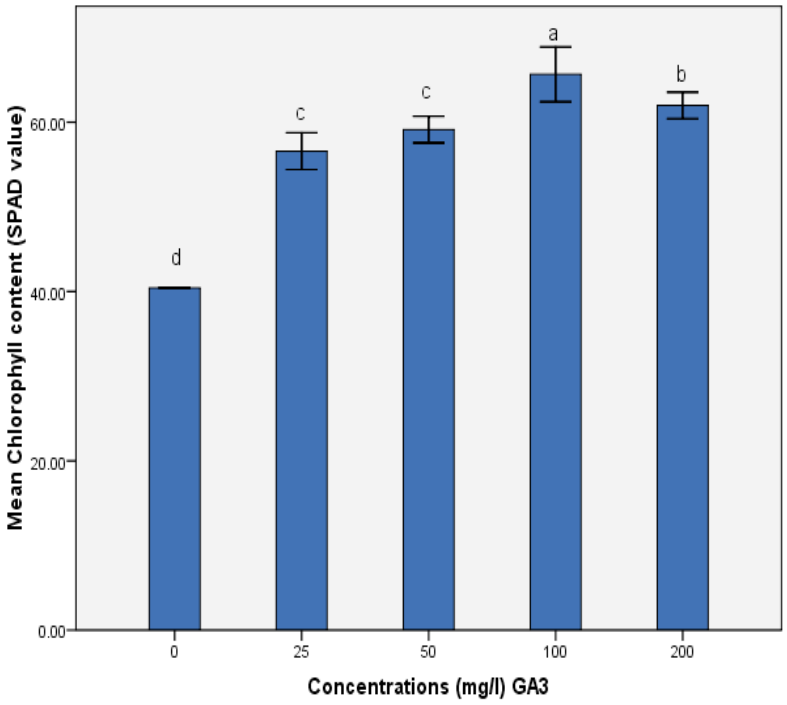

Figure 4.9. Effect of different concentrations of GA_3 applied by stem injection method on chlorophyll content of okra leaves.

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.26. Yield and yield contributing characters of okra as influenced by GA_3 at different concentrations applied by stem injection technique.

Concentrations (mg/l)	No. of pods/plant	Pod length (cm)	Pod diameter (cm)	Pod size (cm^2)	Single pod weight (g)	Aborted seeds/pod (%)
0	9.50±1.29e	4.23±0.01e	1.23±0.02e	5.20±0.08d	2.72±0.02e	4.64±0.01b
25	26.75±0.96d	6.33 ±0.02d	2.33±0.02d	14.74±0.20c	5.25±0.02d	4.85 ±0.01a
50	31.50±1.29c	8.86±0.03c	3.18±0.05c	28.17±0.21b	8.74±0.05c	4.66±0.01b
100	41.00±1.41a	11.28±0.02a	3.67±0.02a	41.39±0.03a	10.10±0.01a	3.77±0.03d
200	37.50±0.57b	10.12±0.03b	3.33±0.02b	33.69±1.58a	9.32±0.02b	4.29±0.01c

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

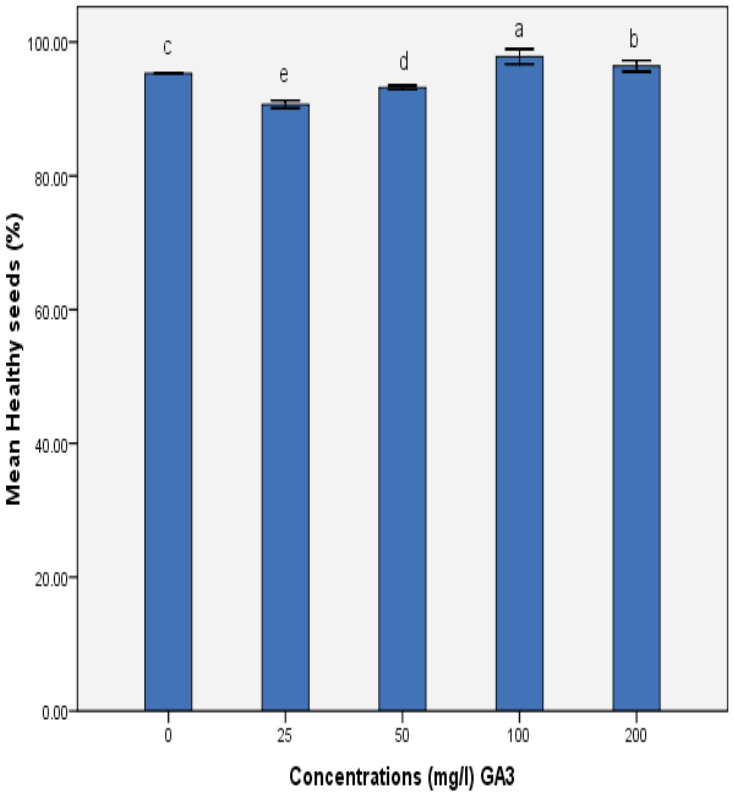

Figure 4.10. Effect of various concentrations of GA_3 on healthy seed percentage per pod (%) applied by stem injection.

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Plate 4.7. Photo shows an effect of 100 mg/l concentration of GA_3 on pod production and pod size applied by stem injection method.

Table 4.27. Difference of flower opening days (blooming) in the treated plants and control under stem injection method with IAA, NAA and GA_3 at various concentrations.

Concentrations	Control	IAA 25 mg/l	IAA 50 mg/l	IAA 100 mg/l	IAA 200 mg/l
Days	57th	57th	57th	57th	57th
Concentrations	Control	GA_3 25 mg/l	GA_3 50 mg/l	GA_3 100 mg/l	GA_3 200 mg/l
Days	57th	55th	50th	40th	43rd
Concentrations	Control	NAA 25 mg/l	NAA 50 mg/l	NAA 100 mg/l	NAA 200 mg/l
Days	57th	57th	57th	57th	57th

4.4 SEED- PRETREATMENT METHODS

Experiment 4

Pre-sowing treatments was applied to okra (*Abelmoschus esculentus*) seeds with Indole acetic acid (IAA); Naphthalene acetic acid (NAA) and Gibberellic acid (GA_3) at different concentrations.

4.4.1 Seed soaked in Indole acetic acid (IAA) and grow them in field (*In vivo*)

4.4.1.1 Seed germination percentage (%)

As showing in Fig. 4.11, pre-sowing treatment of okra seeds with IAA had a beneficial effect on germination percentage at concentration 25 mg/l (96.2 %) as compared to control (78.75 %). However, those seeds that treated with IAA at 50, 100 and 200 mg/l concentrations significantly reduced germination percentage (72.5, 38.8 and 18 %, respectively) as compared with control (78.75 %).

4.4.1.2 Plant height, number of branches, stem girth and number of leaves

Plant height at the end of harvesting stage reflected higher value in IAA 25 mg/l treated plants (84. 25 cm) over control treated plants (75.80 cm) whereas IAA at 50, 100 mg/l and treated plants exhibited higher length of main stem (83.84, 80.94 and 76.34 cm) than control. Number of branches per plant was maximum in plants under IAA 25 mg/l treatment (3.00) followed by 50 mg/l (2.75) and 100 mg/l (2.00) treated seeds. Higher concentration of IAA (200 mg/l) had the lower number of branches (1.50) as compared to control (1.75). Stem girth was highest in 25 mg/l (3.75 cm) while plants of control followed next (2.69 cm). Whereas 200 mg/l treated seeds had lower stem girth (2.10 cm) compared to other treatments. The leaves number was higher in 25 mg/l treated seeds (36.25) over control (19.25). At the same time as, the leave number increased following 50 and 100 mg/l treatment (Table 4.28).

4.4.1.3 Chlorophyll content, Fv/Fm and number of pods

IAA treatments stimulated significant increases in the chlorophyll contents of the okra except 200 mg/l treatment. The highest chlorophyll content (60.75 SPAD value) was obtained under treated seeds with 25 mg/l followed by 50 mg/l (57.21 SPAD value) compared to control (39.75 Spad value). The lowest chlorophyll content (38.62 SPAD value) was given by 200 mg/l pre-sowing treatment. Likewise, the maximum quantum yield of primary photochemistry (Fv/Fm) was significantly increased with application of 25, 50 and 100 mg/l by 31, 27 and 27 % of the control, respectively. Nevertheless, 200 mg/l pre-sowing seed treatment decreased Fv/Fm 12 % in comparison with control (Figure 4.12). Number of pods per plant also responded significantly to IAA applications. The highest number of pods per plant (23.50) was produced by the pre-sowing seed treatment with 25 mg/l, and the lowest (8.50) was given by control plants. A similar trend of the increase in the number of pods per plant also noticed with the application of 50, 100 and 200 mg/l (22, 18 and 11), respectively (Table 4.29).

4.4.1.4 Pod characters and seeds production percentage (%)

The results in table 4.30 showed that pod length was significantly increased by the application of IAA at 25, 50 and 100 mg/l (8.76, 8.67 and 8.52 cm, respectively) compared to control (4.92 cm) whereas, pre-sowing treatment with 200 mg/l decreased it by 17 %. The highest value of pod diameter (3.90 cm) was also obtained from 25 mg/l pre-sowing treatment while the lowest value (1.83 cm) was obtained with 200 mg/l. Generally lower concentration (25 and 50 mg/l) of IAA produced increased the pod size (34.18 and 33.23 cm^2) compared with control (3.62 cm^2) However, statistical analysis on the data showed significant difference of individual pod weight due to application of different rates IAA when applied to the okra

seeds before culturing them. The heaviest pods (10.52g) were observed with 25 mg/l presowing treatment where the lightest pods (3.30) were obtained with 200 mg/l. Also, the results indicated that significant difference was observed on the healthy seed percentage per pod. The Maximum percentage (97.63 %) was produced in 25 mg/l while the lowest being obtained from 200 mg/l (62.62 %).

4.4.1.5 Pods contents of nutritional elements

The data in Table (4.31) indicated that, all the treatments significantly decreased total soluble solids compared to control except 25 mg/l (2.44 % Brixº) as compared with the control (2.37 % Brixº). In addition, pre-sowing treatment of Indole acetic acid (IAA) at 25 mg/l gave the highest values of vitamin C (15.75 mg/100 g) in comparison with the other pre-sowing treatments. A positively high increase in potassium content per pod by IAA at 25, 50 and 100 mg/l presowing treatment was also recorded (97.46, 94.44 and 92.41 mg/100 g), respectively. Even so, 200 mg/l pre-sowing treatment had negatively the effect on TSS, C and K content per pod as compared with control.

With regard to the effect of IAA on pod content of nutritional elements, the data of presented in Table 4.32 indicated that, concentrations of IAA decreased the pod content of Ca, Mg and Na when compared with the control. The least value of Ca, Mg and Na (44.26, 35.25 and 3.92 mg/100 g, respectively) was obtained in the 200 mg/l treatment. But the iron content per pod increased in all IAA pre-sowing treatments except 200 mg/l decreased it relative to control. The 25 mg/l concentration of IAA gave a significantly higher ($P<0.05$) value of 0.463 mg/100 g.

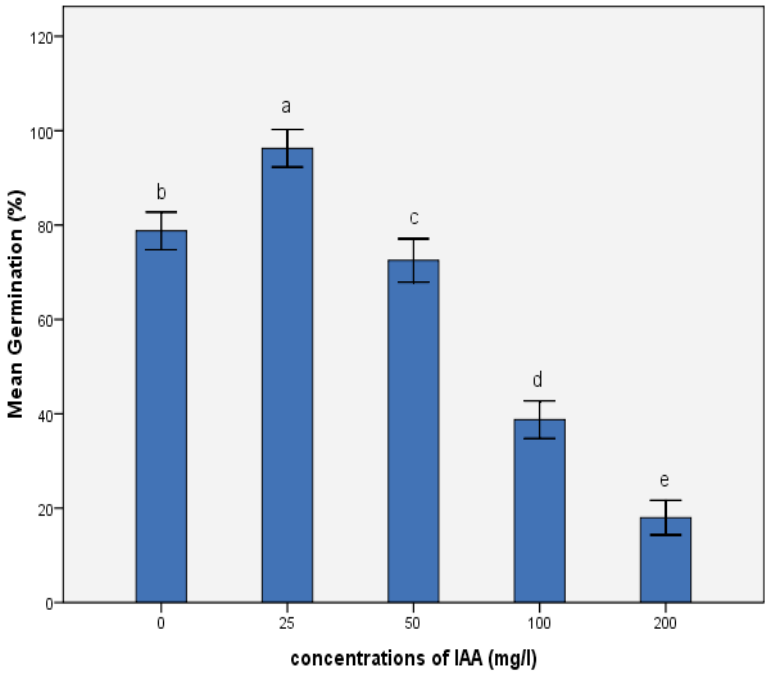

Figure 4.11. Effect of IAA pre-sowing treatment at different concentrations on germination percentage of okra *in vivo* condition.

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.28. Effect of pre-sowing treatment at different concentrations of IAA on vegetative growth parameters of okra *in vivo* condition.

Concentrations (mg/l)	Plant height (cm)	No. of branches/plant	Stem girth (cm)	No. of leaves/plant
0	75.80±0.02e	1.75±0.50c	2.69±0.02b	19.25±0.95d
25	84.25±0.01a	3.00±0.00a	3.75±0.01a	36.25 ±0.50a
50	83.84±0.02b	2.75±0.50a	2.30±0.009c	33.25±1.26b
100	80.94 ±0.05 c	2.00±0.00b	2.28±0.02c	30.00±0.82c
200	76.34±0.01d	1.50±0.57c	2.10±0.02d	19.75±0.96d

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

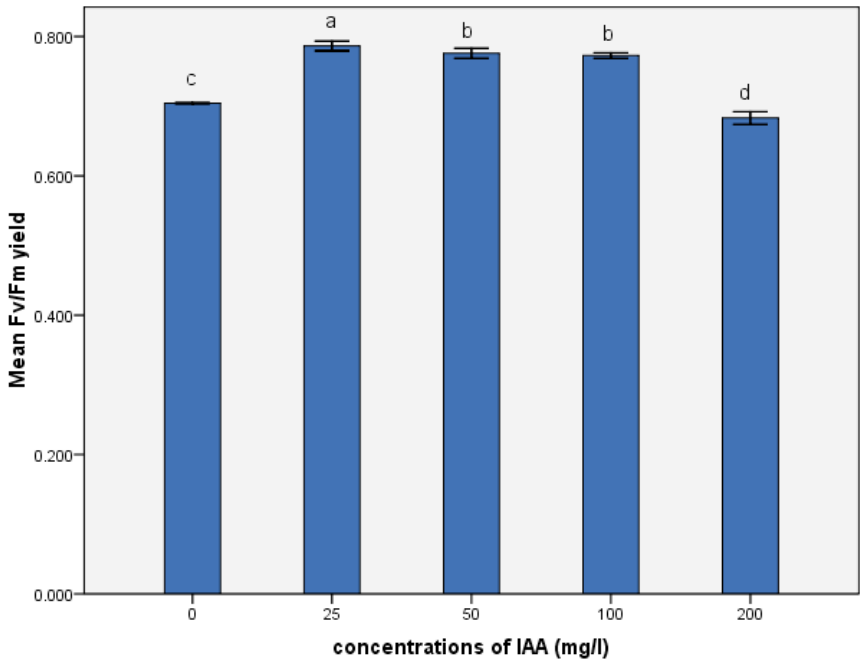

Figure 4.12. The maximum quantum (Fv/Fm) yield of okra leaves at different concentrations of IAA *in vivo* condition.

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.29. Effect of IAA pre-sowing seed treatment on chlorophyll content and pod number of okra plant *in vivo* condition.

Concentrations (mg/l)	Chlorophyll content (SPAD value)	No. of pods/plant
0	39.75±0.50d	8.50±0.57d
25	60.75±0.01a	23.50 ±1.29a
50	57.21±0.02b	22.00±0.82a
100	55.13±0.01c	18.00±1.16b
200	38.62±0.02e	11.00±1.41c

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.30. Yield and yield contributing characters of okra as influenced by IAA at different concentrations *in vivo* condition.

Concentrations (mg/l)	Pod length (cm)	Pod diameter (cm)	Pod size (cm^2)	Single pod weight (g)	Healthy seeds/pod (%)	Aborted seeds/pod (%)
0	4.92±0.01d	2.16±0.01d	8.46±0.08d	3.62±0.01d	93.23±0.02c	6.75±0.01c
25	8.76±0.02a	3.90 ±0.01a	34.18±0.05a	10.52±0.01a	97.63±0.01a	2.35 ±0.01e
50	8.67±0.01b	3.83±0.01b	33.23±0.14b	9.81±0.03b	96.33±0.01b	3.65±0.02d
100	8.52±0.01c	3.53±0.02c	30.14 ±0.10 c	9.42±0.02c	80.16±0.01d	19.83±0.02b
200	4.10±0.02e	1.83±0.03e	7.50±0.14e	3.30±0.03e	62.62±0.01e	37.38±0.02a

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.31. Effect of various concentrations of IAA on total soluble solid, ascorbic acid and K of okra applied by seeds soaking method *in vivo* condition.

Concentrations (mg/l)	Soluble solid (%Brix)	Vit.C (mg/100 g)	K (mg/100 g)
0	2.37±0.01b	10.96±0.02d	92.30±0.02c
25	2.44±0.02a	15.75±0.01a	97.46±0.01a
50	2.36±0.01c	13.56±0.02b	94.44±0.01ab
100	2.36±0.01c	11.53±0.01c	92.41±0.02b
200	2.36±0.01c	8.98±0.02e	88.16±0.02d

Values are mean ± standard deviation

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.32. Nutritional elements (mg/100 g) of okra pod as affected by different concentrations of IAA applied by pre-sowing method *in vivo* condition.

Concentrations (mg/l)	Ca	Mg	Fe	Na
0	59.31±0.01a	39.28±0.01a	0.420±0.01d	6.16±0.01a
25	57.27±0.02b	39.00±0.02b	0.463±0.01a	6.07±0.01b
50	53.22±0.02c	38.56±0.01c	0.451±0.01b	6.02±0.01c
100	50.19±0.02d	37.52±0.01d	0.429±0.01c	5.05±0.02d
200	44.26±0.02e	35.25±0.04e	0.332±0.01e	3.92±0.01e

Values are mean ± standard deviation

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

4.4.2 Seed soaked in Naphthalene acetic acid (NAA) and grow them in field (*In vivo*)

4.4.2.1 Seed germination percentage (%), plant height, number of branches and stem girth

The mean value of germination percentage was computed (Table 4.33). The maximum germination percentage (100 %) was observed using NAA 25 mg/l followed by NAA 50 mg/l in comparison to control (77.5 %) whereas, the minimum germination percentage was observed under NAA 100 mg/l (58 %) followed by 200 mg/l (42 %) in comparison to control and other treatments. Plant height was recorded at the time of last edible pod harvest. It was observed that 25 and 50 mg/l concentrations of NAA exhibited the highly significant effects on the plant height (83.66 and 84.53 cm) compared with control (75.80 cm). Plants grown with high concentration of NAA (200 mg/l) were found to be significantly shortest (72.83 cm) then the plants grown with adequate concentrations of NAA treatments. Plant height increased with the lower rate of NAA up to 50 mg/l as compared to control (77.46 cm). Number of branches per plant varied significantly with variation in NAA doses. The number of branches per plant increased with rate 25 and 50 mg/l application thereby producing the maximum branches (2.50 and 3), respectively. However, application of higher concentration of NAA (200 mg/l) decreased the number of branches per plant (1.25) and 50 mg/l had the maximum number (3.00). It is important to note that increased number of branches plants had led to increase yield of okra. Effect of NAA application was found to be significant on stem girth of plants. It ranged from 2.26 to 3.17 cm.

4.4.2.2 Number of leaves, chlorophyll content and Fv/Fm

The observation recorded in table 4.34 clearly revealed that the number leaves per plant, chlorophyll content and the maximum quantum yield of primary photochemistry (Fv/Fm) differed significantly with each other due to the effect of different concentrations of naphthalene acetic acid (NAA). The maximum number of leaves (40.50), chlorophyll content (53.24 SPAD value) and highest value of Fv/Fm (0.771) were noted under 50 mg/l. The minimum values of all the parameter were noted under the 200 mg/l.

4.4.2.3 Yield and yield contributing

The numbers of pods per plant and pods characters were affected by the treatment of NAA with different concentrations (Table 4.35). Pre-sowing treatment with NAA at 50 mg/l had the highest number of pods per plant (20.50) whereas 200 mg/l pre-sowing treatment had the lowest number of pods (7.50) in comparison with control (8.50). Similarly, 50 mg/l pre-sowing treatment gave the tallest pod length (8.55 cm) while 200 mg/l produced the shortest pod (4.20 cm). In this respect, the largest pod diameter (3.90 cm) was obtained with 50 mg/l application. Pre-sowing treatment with 50 mg/l also gave the greatest pod weight per plant of 9.52g. In addition, the best yield was observed with 25 and 50 mg/l pre-sowing treatment, respectively.

4.4.2.4 Seed production percentage (%)

Okra plants applied with NAA at 25, 50, 100 mg/l concentrations (90.94, 84.30 and 76.10%, respectively) had significantly ($P<0.05$) higher healthy seed percentage than control (93.23 %) and 200 mg/l (62.12) (Figure 4.12). In another word, NAA pre-sowing treatment at

concentrations 25, 50, 100 and 200 mg/l increased aborted seeds percentage compared to control (Figures 4.13 and 4.14).

4.4.2.5 Pod contents of nutritional elements

Results in table 4.36 indicated that pod content of total soluble solids, vitamin C and potassium were affected significantly by different concentrations of NAA. 50 mg/l treatment gave the highest pod content of total soluble solids (2.57 mg/100 g) as compared with (25, 0, 100 and 200 mg/l), respectively. NAA caused a significantly increased in vitamin C content per pod. The highest content per pod (15.41) was in 50 mg/l pre-sowing treatment while the lowest content (10.96 mg/100 g) was observed with control treatment. Also, the highest content of K per pod (98.41 mg/100 g) was found in the treatment of 50 mg/l, but the lowest content (90.20 mg/100 g) was obtained in 200 mg/l. Although, pod content of calcium, magnesium and sodium decreased according to NAA presowing treatment at each concentration, and the highest value of these elements (59.31, 39.28 and 6.16 mg/100 g) was in control pods (Table 4.37). In the contrary, pod content of iron was increased under NAA pre-sowing treatment as compared to control. The maximum increase of iron content (0.441 mg/100 g) was observed at 50 mg/l but there was no significant difference between 200 mg/l (0.421 mg/l) and control (0.420 mg/l).

Table 4.33. Vegetative characters of okra were influenced by pre-sowing seeds with NAA at various concentrations *in vivo* condition.

Concentrations (mg/l)	**Germination (%)**	**Plant height (cm)**	**No. of branches/plant**	**Stem girth (cm)**
0	77.5	75.80±0.02d	1.75±0.50c	2.69±0.02c
25	100	83.66±0.02b	2.50±0.57b	2.92±0.01b
50	83	84.53±0.02a	3.00±0.00a	3.17±0.03a
100	58	77.46 ±0.03c	1.50±0.57d	2.85±0.02b
200	42	72.83±0.01e	1.25±0.50e	2.26±0.01d

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.34. No. of leaves, chlorophyll content and Fv/Fm yield of okra as affected by the pre-sowing application of NAA (25, 50, 100 and 200 mg/l) *in vivo* condition.

Concentrations (mg/l)	No. of leaves/plant	Chlorophyll content (SPAD value)	Fv/Fm yield
0	19.25±0.95d	39.75±0.50d	0.704±0.50d
25	35.50±0.58b	52.70±0.01b	0.761±0.01b
50	40.50±0.57a	53.24±0.01a	0.771±0.02a
100	21.75±0.95c	51.77±0.01c	0.733±0.01c
200	9.00±0.81e	38.30±0.01e	0.603±0.02e

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT)

Table 4.35. Yield and yield contributing characters of okra as influenced by pre-sowing treatment with NAA at different concentrations *in vivo* condition.

Concentrations (mg/l)	No. of Pods/plant	Pod length (cm)	Pod diameter (cm)	Single pod weight (g)
0	8.50±0.57d	4.92±0.01d	2.16±0.01d	3.62±0.01d
25	19.25±0.95b	7.35±0.01b	3.83 ±0.01b	7.43±0.01b
50	20.50±1.29a	8.55±0.01a	3.90±0.01a	9.52±0.03a
100	14.75±0.95c	6.29±0.01c	2.43±0.02c	4.11±0.02c
200	7.50±0.57e	4.20±0.02e	2.09±0.03e	3.42±0.03e

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

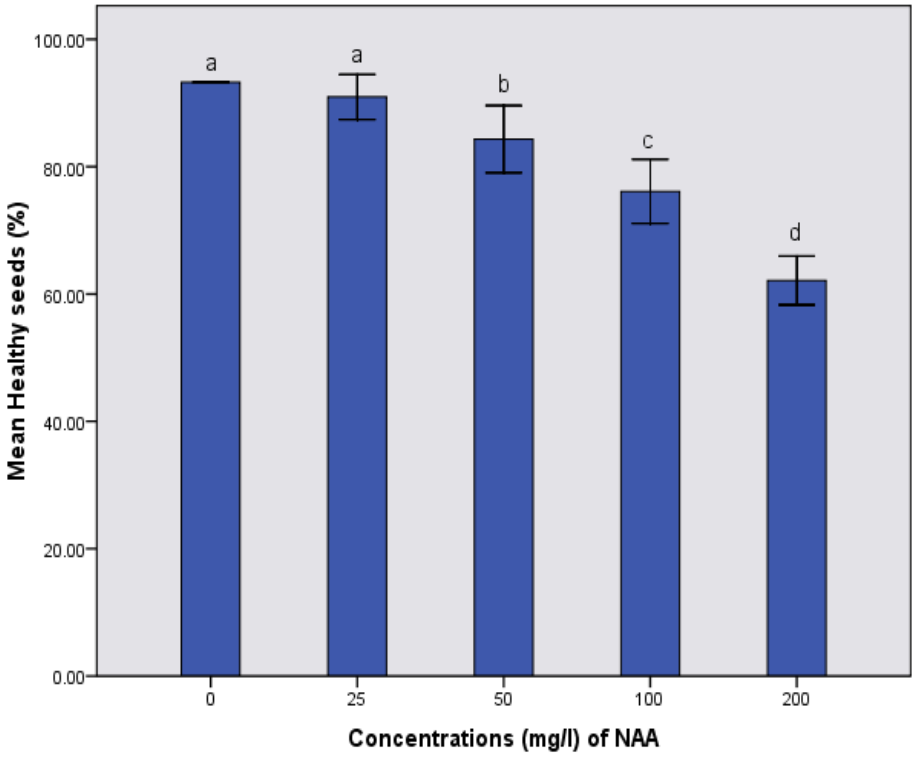

Figure 4.13. Effect of pre-sowing application of NAA on health seed percentage (%) *in vivo* condition.

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

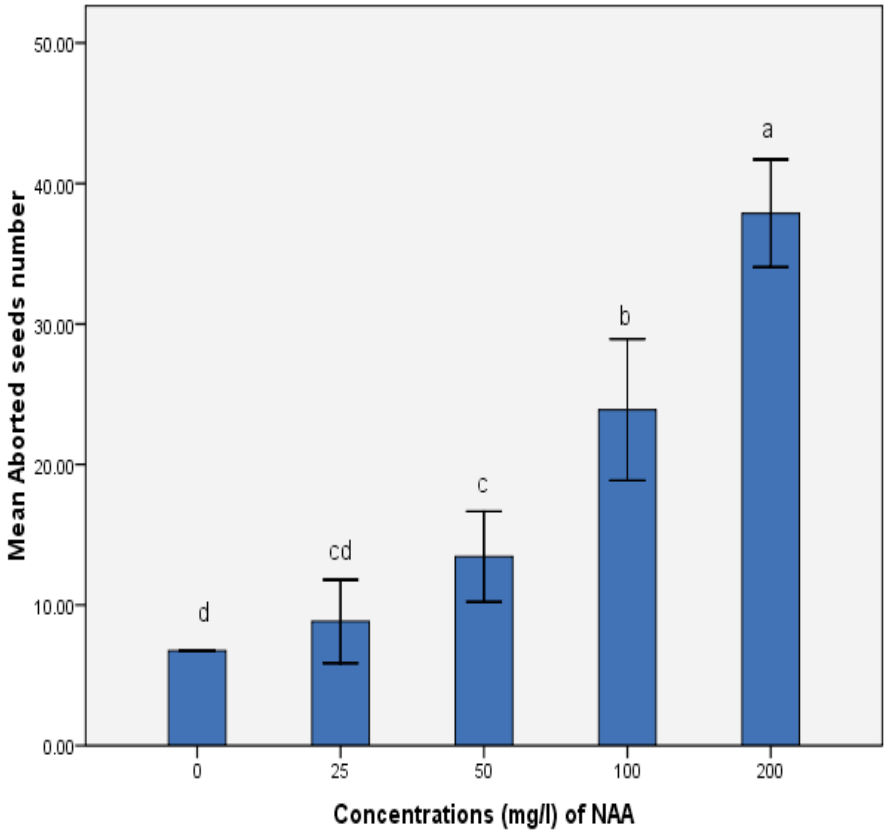

Figure 4.14. Effect of pre-sowing application of NAA on aborted seed percentage *in vivo* condition.

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.36. Effect of different concentrations of NAA on total soluble solid, vitamin C and K content of okra applied by seeds soaking method *in vivo* condition.

Concentrations (mg/l)	TSS (%Brix)	Vit.C. (mg/100 g)	K (mg/100 g)
0	2.37±0.01c	10.96±0.02e	92.30±0.02c
25	2.46±0.01b	12.37±0.02b	95.34±0.01b
50	2.57±0.03a	15.41±0.02a	98.41±0.01a
100	2.21±0.01d	11.93±0.01c	92.31±0.01c
200	2.25±0.01e	11.84±0.01d	90.20±0.01d

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.37. Analysis of nutritional elements (mg/100) of *A. esculentus* pod as affected by various concentrations of NAA applied by pre-sowing treatment *in vivo* condition.

Concentrations (mg/l)	Ca	Mg	Fe	Na
0	59.31±0.01a	39.28±0.01a	0.420±0.01c	6.16±0.01a
25	57.34±0.01ab	39.27±0.02a	0.430±0.02b	6.04±0.01b
50	56.03±0.01bc	39.05±0.01b	0.441±0.01a	6.02±0.02b
100	54.83±0.02bc	38.25±0.01c	0.427±0.02b	5.97±0.02c
200	52.86±0.01c	32.29±0.01d	0.421±0.01c	5.81±0.01d

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

4.4.3 Seed soaked in Gibberellic acid (GA_3) and grow them in field

4.4.3.1 Seed germination percentage, Plant height, number of branches, and stem girth

The observations of the experiment showed that GA_3 25 and 50 mg/l resulted in equal 97.5% of germination, which followed by 100 and 200 mg/l while control set showed the lowest germination (77.5 %) figure 4.15.

The results in Table 4.38 indicated that plant height of okra plant was significantly influenced by the application of GA_3. The highest plant (89.84 cm) was recorded from the highest dose of GA_3 that is 200 mg/l. The second highest plant (87.67 cm) was noted from 100 mg/l, which was statistically different than 50 and 25 mg/l (85.25 and 83.54 cm), while the shortest plant (75.80 cm) was observed in control. On another hand, application of lower and higher concentrations of GA_3 increased the number of branches. However, the Maximum number was with 200 mg/l (3.00). Also, the stem girth of plants increased linearly with increased rate of GA_3 application. Treatment with 200 mg/l induced greater stem girth (5.21 cm) followed by 100 mg/l (4.16 cm) and 50 mg/l (2.98 cm) while the lowest stem girth value was obtained in control plants.

4.4.3.2 Number of leaves, chlorophyll content and Fv/Fm

Average values for different leaf parameters (Table 4.39) was revealed that the pre-sowing treatment with GA_3 at different levels increased the number of leaves as well as chlorophyll content and Fv/Fm yield, whereas the seeds treated with 200 mg/l produced the maximum (47.50) number of leaves, highest content (66.18 SPAD value) of chlorophyll and highest quantum yield of primary photochemistry (Fv/Fm) (0.802). The results indicated that the

control treatment registered significantly lowest values of leave parameters (19.25, 325.73 cm, 39.75 SPAD value and 0.604) respectively.

4.4.3.3 Yield and yield contributing and seeds production

It was observed from the data in table 4.40 that number of pods and pod contributing greatly affected with pre-sowing treatment with GA_3 at different concentrations and that treated with GA_3 at 100 and 200 mg/l produced the highest number of pods, which reached 23.75, followed by 50 and 25 mg/l treatments, which produced 20.59 pods. The control treatment produced the lower number of pods than the above treatments (8.50). Pod length and pod diameter were also affected with the GA_3 treatments. Highest pod length was recorded at 200 mg/l (10.97 cm) and the lowest at control treatment (4.29 cm). Similarly, the highest value of diameter was at 200 mg/l (4.05 cm); this was, however, closely followed by 100 mg/l treatment (3.89 cm). In this context, 200 mg/l pre-sowing treatment produced the heaviest pods (15.42g) followed by 100 mg/l (14g) while control plants produced the lightest pods (3.61g). Gibberellic acid pre-sowing treatment of okra seeds significantly increased healthy seeds percentage compared to control with pre-sowing treatment. The production of healthy seed percentage per pod increased with GA_3 application (Figure 4.16).

4.4.3.4 Pods contents of nutritional elements

Seed soaking with GA_3 had a significant effect on pod contents of total soluble solids, vitamin C and potassium (Table 4.41). In the GA_3 pre-sowing treatment, the relatively increase in total soluble solids (TSS). It was significantly more in higher concentrations (100 and 200 mg/l) than control. The increase in TSS was, in general dependent on the concentration of GA_3. At higher concentrations increase was more, while at the lower concentrations increase was less, maximum being at highest concentration (200 mg/l) (2.77

mg/100 g) as compared to control (2.37 mg/100 g). However, there was no significant difference between control, 25 and 50 mg/l. In addition, the pod content of vitamin C showed a remarkable increase with applied GA_3. At lower concentrations (25 and 50 mg/l) increasing was less (12.76 and 12.91mg/100 g, respectively) than higher concentrations (100 and 200 mg/l) but it was higher than control (10.96 mg/100 g). Moreover, all concentrations of GA_3 increased the pod content of potassium except 25 mg/l (92.54 mg/100g) did not show any significant effect as compared to control (92.30 mg/100g). The highest value of potassium (101.14 mg/100 g) was obtained with 200 mg/l treatment compared to other treatments.

As shown in table 4.42, pre-sowing treatment of seeds with GA_3 at different concentrations decreased the pod contents of calcium (Ca) and iron (Fe) compared with control. Maximum pod contents of Ca and Fe (59.31 and 0.420 mg/100 g) was achieved from control pods followed by 25 mg/l (58.26 and 0.406 mg/100 g) treatment. Minimum pod contents of both were observed in 200 mg/l treatment (55.22 and 0.373 mg/100 g). Magnesium content was also significantly affected by different concentrations of GA_3 compared with control. The pre-sowing treatments of seeds decreased the pod content of magnesium as a whole compared with control (39.28 mg/100 g). Maximum pod content of magnesium (39.28 mg/100 g) were recorded from control pods, statistically similar pod content of magnesium in 25 and 50 mg/l. But high concentrations of GA_3 (100 and 200 mg/l) decreased remarkably compared to other treatments (39.20 and 38.75 mg/100) respectively. Contrary to negative effect of pre-sowing treatment with GA_3 on mentioned elements, GA_3 had a positive effect on pod content with Na where all of treatments increased it in comparison with control (6.16 mg/100 g). At the high concentrations (100 and 200 mg/l) Na content increased and Na was significantly higher

(6.45 and 6.62 mg/100 g) than lower concentrations (25 and 50 mg/l) was less (6.25 and 6.30 mg/100 g), but it was higher than control.

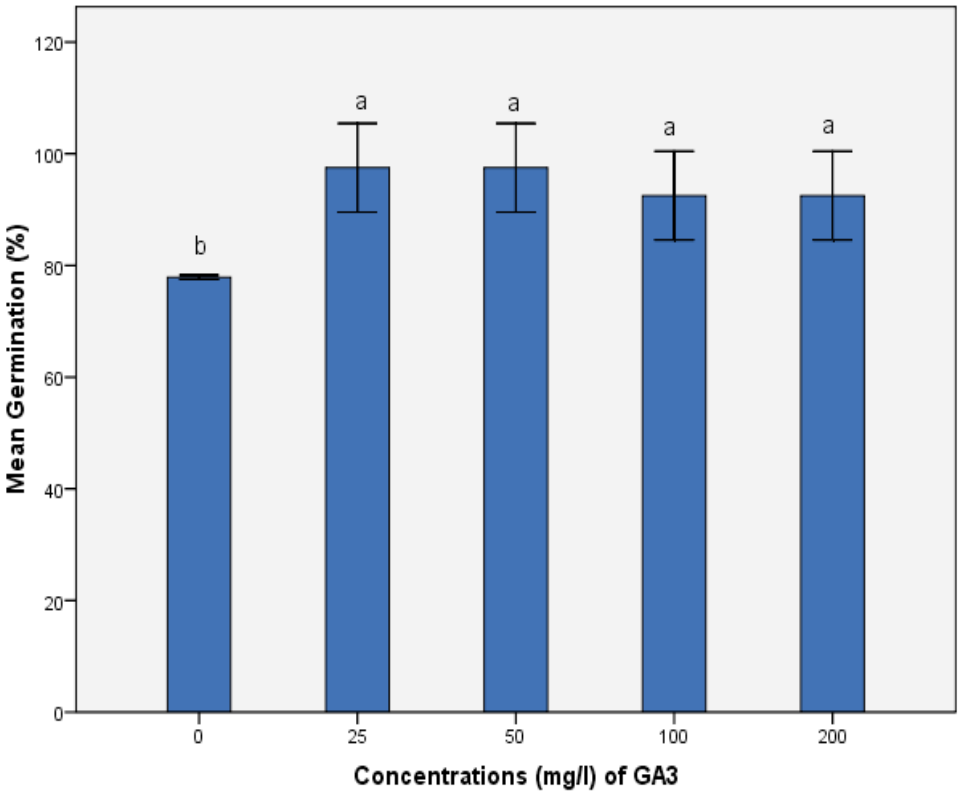

Figure 4.15. Effect of GA_3 pre-sowing treatment at different concentrations on the germination percentage of okra *(Abelmoschus esculentus) in vivo* condition.

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.38. Measurement of plant height, no. of branches and stem girth under pre-sowing treatment with GA_3 at different concentrations *in vivo* condition.

Concentrations (mg/l)	Plant height (cm)	No. of branches/plant	Stem girth (cm)
0	75.80±0.02e	1.75±0.50d	2.69±0.02e
25	83.54±0.02d	200±0.57c	2.79±0.01d
50	85.25±0.02c	2.00±0.50c	2.98±0.02c
100	87.67 ±0.01b	2.50±0.00b	4.16±0.02b
200	89.84±0.02a	3.00±0.00a	5.21±0.01a

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.39. No. of leaves, chlorophyll content and Fv/Fm (maximum quantum yield) of okra leaves as affected by the pre-sowing application of GA_3 at different concentrations *in vivo* condition.

Concentrations (mg/l)	No. of leaves/plant	Chlorophyll content (SPAD value)	Fv/Fm yield
0	19.25±0.95e	39.75±0.50e	0.704±0.50d
25	34.00±0.82d	54.91±0.01d	0.773±0.01c
50	43.25±0.95c	56.71±0.01c	0.771±0.01c
100	45.50±0.58b	62.44±0.01b	0.787±0.01b
200	47.50±0.58a	66.18±0.01a	0.802±0.01a

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.40. Measurement of pod length, pod diameter and pod weight under seed soaking treatment with GA_3 in various concentrations *in vivo* condition.

Concentrations (mg/l)	No. of pods/plant	Pod length (cm)	Pod diameter (cm)	Single pod weight (g)
0	8.50±0.57c	4.29±0.01e	2.16±0.01e	3.62±0.01e
25	20.50±1.92b	8.12±0.01d	3.67±0.02d	9.22 ±0.01d
50	20.59±0.57b	8.64±0.01c	3.78±0.01c	9.80±0.03c
100	23.75 ±0.95a	10.89±0.01b	3.89±0.02b	14.00±0.02b
200	23.75±1.25a	10.97±0.01a	4.05±0.01a	15.42±0.02a

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

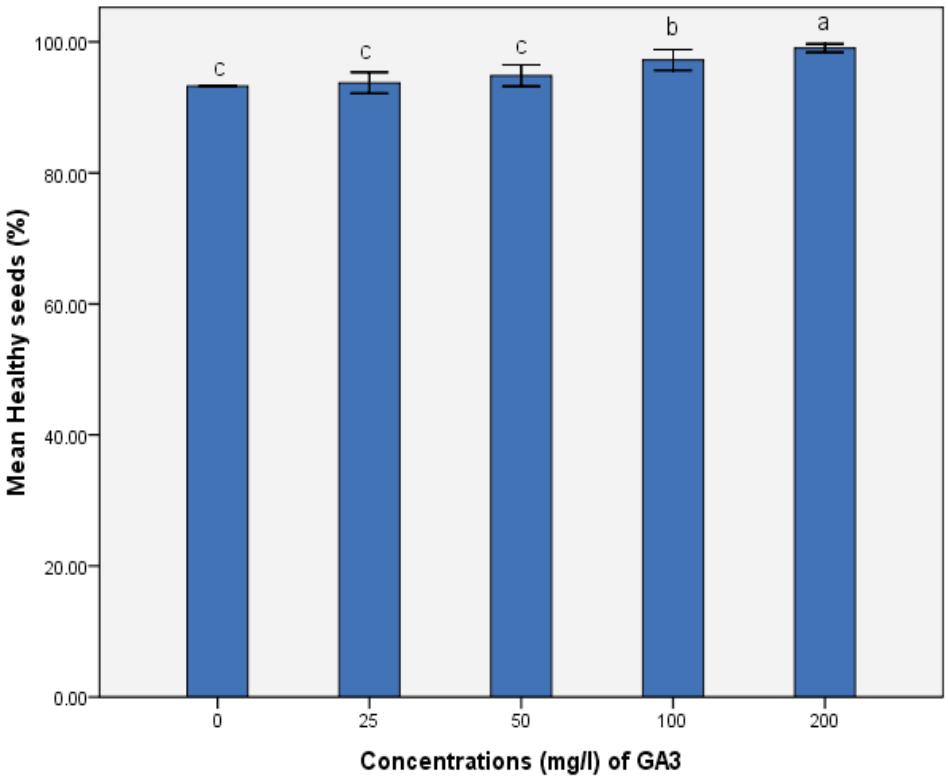

Figure 4.16. The effect of different concentrations of GA_3 on healthy seed percentage under pre-sowing treatment.

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.41. Effect of pre-sowing okra seeds with GA_3 on total soluble solid, vitamin C and K content of pods (mg/100 g) *in vivo* condition.

Concentrations (mg/l)	TSS (%Brix)	Vit.C (mg/100 g)	K (mg/100 g)
0	2.37±0.01bc	10.96±0.02e	92.30±0.02c
25	2.37±0.01bc	12.76±0.01d	92.54±0.01c
50	2.36±0.03c	12.91±0.02c	96.00±0.01b
100	2.49±0.01b	13.46±0.01a	96.64±0.01b
200	2.77±0.03a	13.31±0.01b	101.14±0.01a

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.42. Determination of nutritional elements (mg/100) of *A. esculentus* pods as affected by various concentrations of GA_3 applied by pre-sowing treatment *in vivo* conditions.

Concentrations (mg/l)	Ca	Mg	Fe	Na
0	59.31±0.01a	39.28±0.01a	0.420±0.01a	6.16±0.01e
25	58.26±0.04b	39.28±0.02ab	0.406±0.01b	6.25±0.02d
50	58.12±0.02c	39.25±0.01ab	0.400±0.01c	6.30±0.01c
100	57.20±0.02d	39.20±0.02b	0.387±0.02d	6.45±0.02b
200	55.22±0.01e	38.75±0.01c	0.373±0.01e	6.62±0.01a

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT

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4.4.4 Comparison of duration of germination and flowering time between different concentrations of IAA, NAA and GA_3

4.4.4.1 The duration of germination

Table 4.43 showed the effect of growth regulators on duration of germination. Early germination was observed with GA_3 pre-sowing treatment at 100 and 200 mg/l (five days) as compared to control (seven days) while the IAA and NAA pre-sowing treatment with lower concentrations (25 and 50 mg/l) germination was delayed for two days when compared with control. However, the higher concentrations of IAA and NAA (100 and 200 mg/l) proved to be the most inhibitory, because germination was delayed for six days as compared to control.

4.4.4.2 Flowering

Flowering time was influenced by pre-sowing treatment of IAA, NAA and GA_3 at various concentrations (Table 4.44). Early flowering was obtained with GA_3 at 100 mg/l (42 days) followed by GA_3 at 200 mg/l (46) and IAA at 25 and 50 mg/l. (50) in comparison with control (55) but there was no significant effect of GA_3 treatments at 25 and 50 mg/l. In this respect, flowering was attained in pre-sowing of NAA at 25 and 50 mg/l within 52 days after planting while IAA and NAA applications at higher concentrations (100 and 200 mg/l) delayed the onset of flowering by more than 3 days.

Table 4.43. Effect of IAA, NAA and GA_3 with different concentrations on the duration of germination on treated and control seeds of okra.

Days	**Control**	**IAA 25 mg/l**	**IAA 50 mg/l**	**IAA 100 mg/l**	**IAA 200 mg/l**
	7	9	9	13	13
Days	**Control**	**GA_3 25 mg/l**	**GA_3 50 mg/l**	**GA_3 100 mg/l**	**GA_3 200 mg/l**
	7	7	7	5	5
Days	**Control**	**NAA 25 mg/l**	**NAA 50 mg/l**	**NAA 100 mg/l**	**NAA 200 mg/l**
	7	9	9	13	13

Table 4.44. Difference of flower opening days (blooming) in the treated and control plants under pre-sowing treatment with IAA, NAA and GA_3 at various concentrations *in vivo* condition.

	Control	**IAA 25 mg/l**	**IAA 50 mg/l**	**IAA 100 mg/l**	**IAA 200 mg/l**
Days	55	48	52	58	58
	Control	**GA_3 25 mg/l**	**GA_3 50 mg/l**	**GA_3 100 mg/l**	**GA_3 200 mg/l**
Days	55	55	55	42	46
	Control	**NAA 25 mg/l**	**NAA 50 mg/l**	**NAA 100 mg/l**	**NAA 200 mg/l**
Days	55	52	52	58	58

4.5 GROW SOAKED SEEDS ON MURASHIGE AND SKOOG'S MEDIUM (*IN VITRO*)

Experiment 5

Effect of Indole acetic acid (IAA); Naphthalene acetic acid (NAA) and Gibberellic acid (GA_3) at different concentrations.

4.5.1 Indole acetic acid (IAA)

4.5.1.1 Seed germination percentage, Plant height, number of branches, stem girth and number of leaves

Data in Fig. 4.17 showed that germination percentage was higher in 25 and 50 mg/l IAA treated plant compared to control (0 conc.). Moreover, IAA treatments with the concentrations 100 mg/l had a lower pod setting percentage compared to 0, 25 and 50 mg/l. But treatment with IAA at 200 mg/l concentration inhibited the seed germination (0%). The best concentration was found 25 and 50 mg/l (Plate 4.8).

The highest plant height was obtained 79.35 cm in 50 mg/l, and the shortest plants were found 73.93 cm in control (0 mg/l) (Table 4.45). The maximum number of branches was (3) in 50 mg/l whereas the lowest branches were obtained with control treatment (1.75) per plant. Stem girth was 2.96, 2.80, 2.67 and 2.54 cm in 50, 100, 25 and 0 mg/l respectively. Number of leaves was observed higher in 50 mg/l IAA than other all concentrations and control. It can be seen in table 4.45 the highest leave number were 31.50 with 50 mg/l IAA treatment, and the lowest number was with control treatment (18.75).

4.5.1.2 Chlorophyll content, Fv/Fm yield and number of pods

Results showed that chlorophyll content, Fv/Fm yield and number of pods per plant were higher in 50 mg/l treated concentration followed by 100, 25 and 0 mg/l concentrations (Table 4.46). Chlorophyll content was higher in all IAA concentrations compared with control (0 mg/l) except 200 mg/l. In this respect, the highest Fv/Fm yield was observed with 50 mg/l (0.783) as compared to control treatment (0.602). Also, pre-sowing treatment with 50 mg/l produced the highest number of pods (20.75) when compared to other treatments.

4.5.1.3 Pod growth, development and seed production

Data in table 4.47 showed that the maximum pod length was obtained 7.22 cm in 50 mg/l, and the minimum was found 3.24 cm in control (0 mg/l). In this respect, the maximum pod diameter was 1.94 cm in 50 mg/l whereas the minimum was observed by control treatment (1.26 cm). Per pod, weight was 8.33, 7.13, 4.83, and 2.75g in 50, 25, 100 and 0 mg/l respectively. Soaked seeds with 50 mg/l gave the biggest pod size (14.02 cm^2) while control treatment gave the smallest pod size (4.10 cm^2). 86.15% healthy seed was found in 0 mg/l IAA while 52.54% healthy seed was recorded in 100 mg/l. Pre-sowing treatment with IAA at 100 mg/l produced the highest aborted percentage (47.45%) and pre-sowing treatment with 0 mg/l produced the lowest aborted seed's percentage (13.84%) (Figure 4.18).

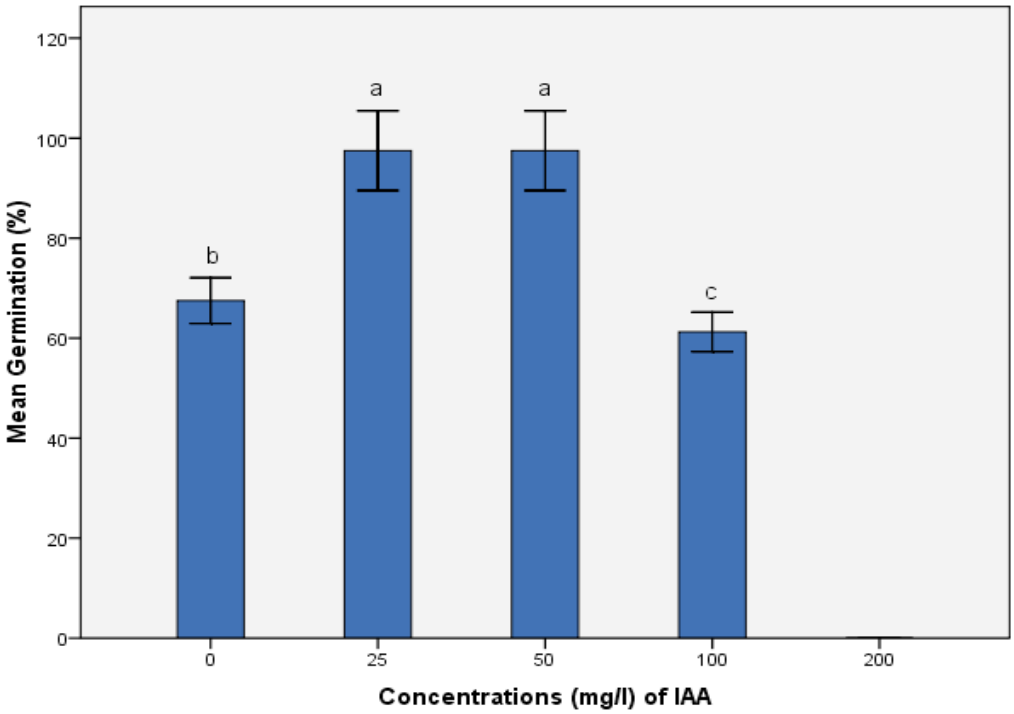

Figure 4.17. Effect of IAA at various concentrations on the germination percentage of okra seeds under *in vitro* condition.

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

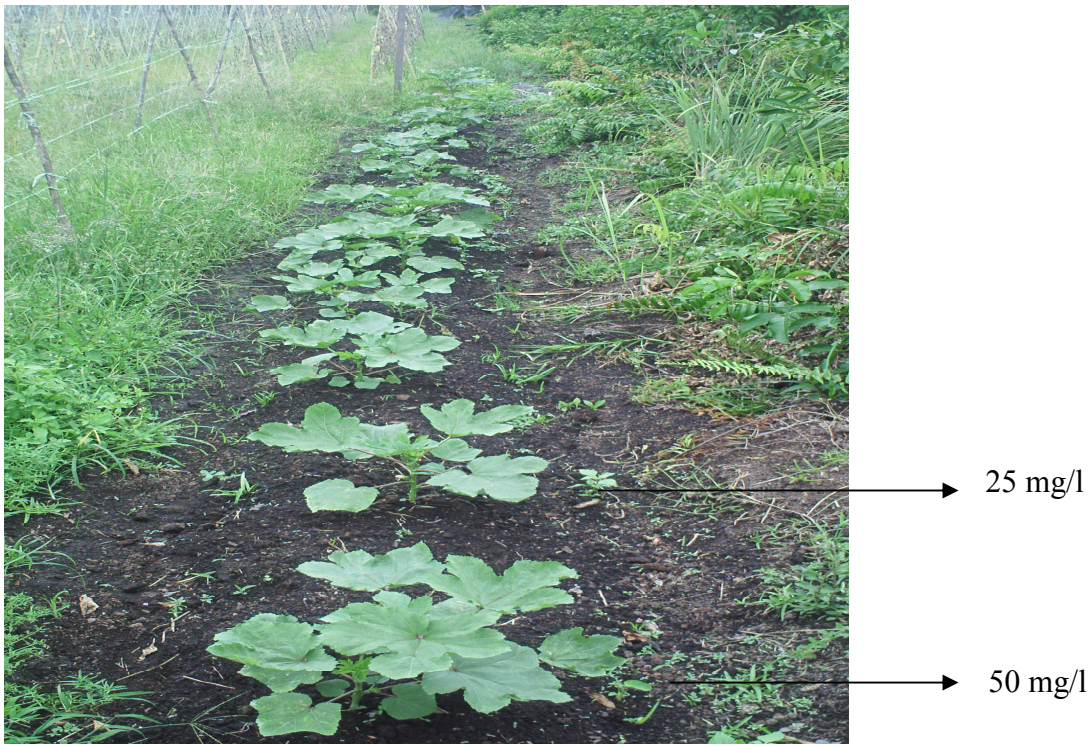

Plate 4.8. Growth of okra plants from seed treatment with 25 and 50 mg/l under *in vitro* condition.

Table 4.45. Effect of pre-sowing treatment with different concentrations of IAA on vegetative growth parameters of okra under *in vitro* condition.

Concentrations (mg/l)	Plant height (cm)	No. of branches/plant	Stem girth (cm)	No. of leaves/plant
0	73.93±0.04d	1.75±0.50c	2.54±0.01d	18.75±0.95c
25	77.39±0.01c	2.25±0.50bc	2.67±0.01c	27.00 ±0.82b
50	79.35±0.01a	3.00±0.50a	2.96±0.01a	31.50±1.92a
100	78.94 ±0.01b	2.50±0.57ab	2.80±0.01b	28.75±1.50b
200	0	0	0	0

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).
0: No germination

Table 4.46. Measurement of chlorophyll content, Fv/Fm yield and number of pods per plant using pre-sowing treatment with IAA at different concentrations (mg/l) in okra plants under *in vitro* condition.

Concentrations (mg/l)	Chlorophyll content (SPAD value)	Fv/Fm yield	Number of pods/plant
0	39.75±0.50d	0.702±0.01d	8.50±0.76d
25	43.87±0.10c	0.775±0.01c	17.50±1.00c
50	53.23±0.02a	0.783±0.01a	20.75±0.96a
100	52.67±0.02b	0.777±0.01b	19.00±0.816b
200	0	0	0

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).
0: No germination

Table 4.47. Effect of pre-sowing treatment of IAA at different concentrations on pod characters and healthy seeds percentage of okra plant under *in vitro* condition.

Concentrations (mg/l)	Pod length (cm)	Pod diameter (cm)	Single pod weight (g)	Pod size (cm^2)	Healthy seeds (%)
0	4.24±0.01d	1.26±0.01d	2.75±0.02d	4.10±0.04d	86.15±0.02a
25	5.11±0.01b	1.56±0.02b	7.13±0.02b	7.99±0.08b	83.14±0.01a
50	7.22±0.02a	1.94±0.01a	8.33±0.01a	14.02±0.10a	76.91±0.02c
100	4.13±0.01c	1.43±0.01c	4.83±0.01c	5.89±0.10c	58.54±0.02d
200	0	0	0	0	0

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).
0: No germination

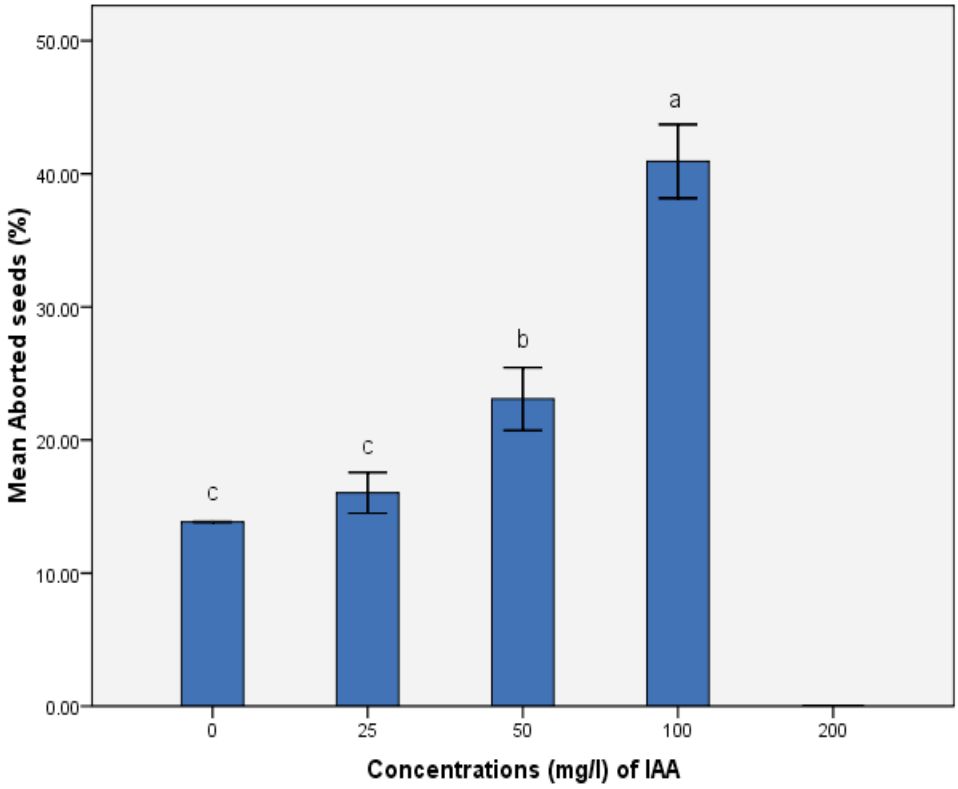

Figure 4.18. Aborted seed percentage using pre-sowing treatment with IAA at different concentrations of okra under *in vitro* condition.

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

4.5.2 Naphthalene acetic acid (NAA)

4.5.2.1 Seed germination percentage, Plant height, number of branches and stem girth

The mean value of germination percentage was computed (Table 4.48). The maximum germination percentage (100%) was observed through NAA 25 mg/l followed by NAA 50 mg/l (95.75%) in comparison to control set (67.5%) whereas, the minimum germination percentage was observed under NAA 100 mg/l (42.5%) followed by 200 mg/l (0%) in comparison to control and other treatments.

Plant height was recorded at the time of last edible pod harvest. It was observed that 25 mg/l level of NAA exhibited a highly significant effects on the plant height (79.86 cm) compared with control (73.93 cm). Number of branches per plant varied significantly with variation in NAA concentrations. The number of branches per plant increased with rate 25 and 50 mg/l application thereby producing the maximum branches (2.75 and 2.50) under application of 25 and 50 mg/l, respectively. The minimum number of branches (1.75) was recorded at 100 and 0 mg/l. It is important to note that increased number of branches plants had led to increase yield of okra. Effect of NAA application was found to be significant on stem girth of plants. It ranged from 2.54 to 2.71 cm.

4.5.2.2 Number of leaves, chlorophyll content and Fv/Fm

The observation recorded in table 4.49 clearly revealed that the number leaves per plant, chlorophyll content and the maximum quantum yield of primary photochemistry (Fv/Fm) differ significantly with each other due to the effect of different doses of naphthalene acetic acid (NAA). The maximum number of leaves 30.50, chlorophyll content 53.77 SPAD value

and highest value of Fv/Fm 0.781 were noted under 25 mg/l. The minimum values of all the parameter were noted under the control treatment (0 mg/l).

4.5.2.3 Pod growth, development and seed production

The number of pods per plant and pods character was affected by the treatment of NAA with different concentrations (Table 4.50). Pre-sowing treatment with NAA at 25 mg/l under *in vitro* condition had the highest number of pods per plant (19.50) whereas control treatment (0 mg/l) pre-sowing treatment had the lowest number of pods (8.50). Also, 25 mg/l pre-sowing treatment gave the highest pod length (8.26 cm) in comparison to control treatment (4.24 cm). The largest pod diameter (2.13 cm) was obtained with 25 mg/l application as compared to control (1.26 cm). Pre-sowing treatment with 25 mg/l gave the greatest pod weight per plant of 9.16g. Okra plants applied with NAA at 25 mg/l concentrations had significantly ($P<0.05$) higher healthy seed percentage (89.2 %) than control (86.1%), 50 (86.2) and 100 mg/l (45.1%). In another word, 54.9% aborted seed was recorded in 100 mg/l pre-sowing treatment in comparison with control treatment as shown in Figure 4.19 and Plate 4.9.

Table 4.48. Effect of pre-sowing treatment applied by NAA on seed germination, plant height, number of branches and stem girth of okra plant under *in vitro* condition.

Concentrations (mg/l)	Germination (%)	Plant height (cm)	No. of branches/plant	Stem girth (cm)
0	67.50±2.89c	73.93±0.04d	1.75±0.50b	2.54±0.01c
25	100.00±0.00a	79.86±0.01a	2.75±0.50a	2.71 ±0.02a
50	95.75±2.98b	76.91±0.02b	2.50±0.58a	2.71±0.01a
100	42.50 ±2.89d	76.67±0.02c	1.75±0.50b	2.57±0.02b
200	0	0	0	0

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).
0: No germination

Table 4.49. No. of leaves, chlorophyll content and Fv/Fm yield of okra leaves as affected by the pre-sowing application of NAA (25, 50, 100 and 200 mg/l) under *in vitro* condition.

Concentrations (mg/l)	No. of leaves/plant	Chlorophyll content (SPAD value)	Fv/Fm yield
0	18.75±0.95d	39.75±0.50d	0.702±0.01d
25	30.25±1.25a	53.77±0.01a	0.781±0.01a
50	25.25±1.25b	53.13±0.01b	0.779±0.01b
100	23.25±1.50c	42.95±0.01c	0.768±0.02c
200	0	0	0

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).
0: No germination

Table 4.50. Yield and yield contributing characters of okra as influenced by pre-sowing treatment with NAA at different concentrations under *in vitro* condition.

Concentrations (mg/l)	No. of Pods/plant	Pod length (cm)	Pod diameter (cm)	Single pod weight (g)
0	8.50±0.76c	4.24±0.01d	1.26±0.01d	2.75±0.02d
25	19.50±0.96a	8.26±0.02a	2.13 ±0.01a	9.16±0.02a
50	18.50±1.30a	8.21±0.01b	2.08±0.02b	9.09±0.03b
100	14.75±1.30b	7.80±0.02c	2.06±0.03c	9.04±0.02c
200	0	0	0	0

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).
0: No germination

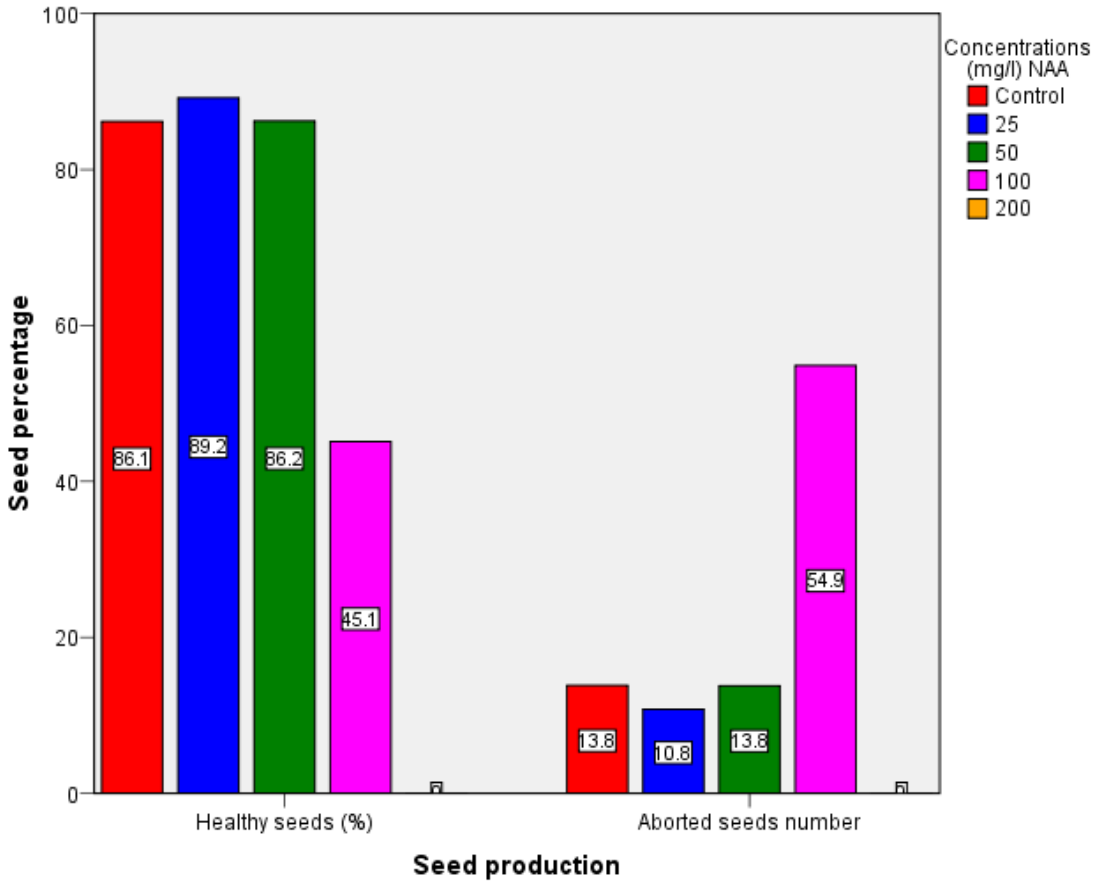

Figure 4.19. Effect of pre-sowing application of NAA on seeds yields percent under *in vitro* condition.

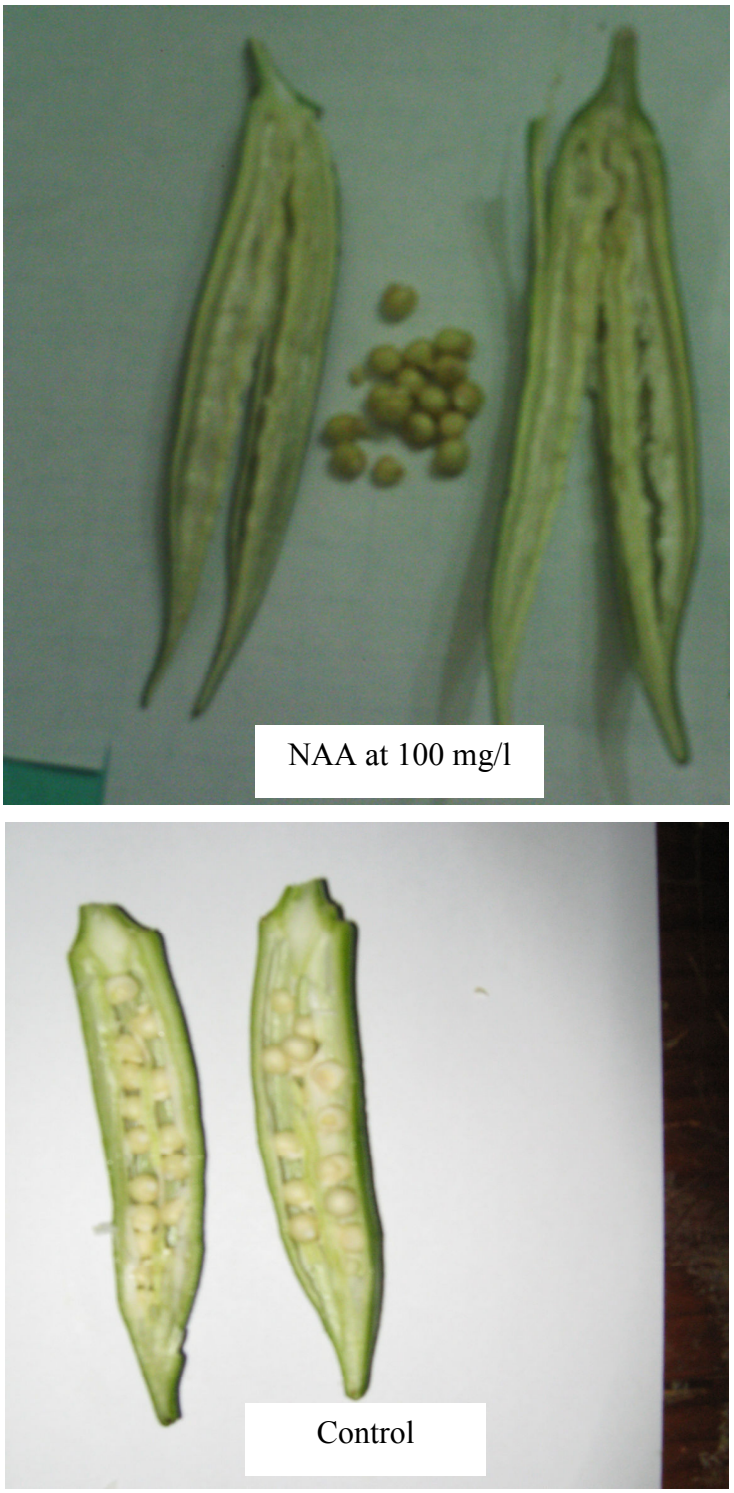

Plate 4.9. Effect of NAA at 100 mg/l on seed production of okra applied by pre-sowing treatment under in vitro condition as compared to control treatment.

4.5.3 Gibberellic acid (GA_3)

4.5.3.1 Seed germination percentage, Plant height, number of branches, stem girth and number of leaves

Seed germination of okra as affected by GA_3 treatments was illustrated in figure 4.20. The maximum germination percentage (100%) was observed through GA_3 at 100 and 200 mg/l followed by GA_3 50 mg/l (98.75%) and 25 mg/l (97.50%) in comparison to control treatment (67.50%).

Results in table 4.51 indicated that GA_3 application at different concentrations under *in virto* condition significantly increased the plant height, branch number, stem girth and number of leaves per plant of *A. esculentus* as compared with untreated seeds (control). The highest plant height value was obtained with GA_3 at 200 mg/l (95.15 cm) whereas the lowest value was observed with control treatment (73.93 cm). Treatments of 25 and 50 mg/l of GA_3 were not significantly different in their effects on the number of branches (1.75) but 100 and 200 mg/l increased the branch number (2.50 and 3) in comparison to control (1.75). Gibberellic acid (GA_3) treatments significantly increased the stem girth of okra compared to the control during study (Table 4.51). The trend was found to be the same as in-plant height. Maximum increase in stem girth was obtained at concentration of 200 mg/l GA_3 treatment of okra (2.81 cm). Significant increases in the number of leaves of okra were recorded in all GA_3 treatments as compared to untreated control (Table 4.51). These increases were higher when 100 and 200 mg/l of GA_3 treatments (29.25 and 34.75).

4.5.3.2 Chlorophyll content, Fv/Fm yield and number of pods

Results showed that chlorophyll content, Fv/Fm yield and number of pods per plant were higher in 200 mg/l treated concentration followed by 100, 50 and 25 mg/l concentrations (Table 4.52). Chlorophyll content was higher in all GA_3 concentrations compared with control (0 mg/l). In this respect, the highest Fv/Fm yield was observed with 200 mg/l (0.796) as compared to control treatment (0.602). Also, pre-sowing treatment with 200 mg/l produced the highest number of pods (27.75) when compared to other treatments.

4.5.3.3 Pod growth, development and seed production

Data in table 4.53 showed that the maximum pod length was observed 7.56 cm in 200 mg/l, and the minimum was found in 25, 50 and control (4.24 cm). In this respect, the maximum pod diameter was observed in 200 mg/l (2.25 cm) whereas the minimum was obtained with 25, 50 mg/l and control treatment (1.26 cm). Per pod, weight was 8.66, 8.39, 2.76, and 2.75g in 200, 100, 50, 25 and 0 mg/l respectively. Soaked seeds under *in virto* condition with 200 mg/l gave the biggest pod size (17.01cm²) while 25 mg/l and control treatments gave the smallest pod size (4.10 cm²). 86.15% healthy seed was found in 0 mg/l GA_3 while 97.14 % healthy seed was recorded in 200 mg/l.

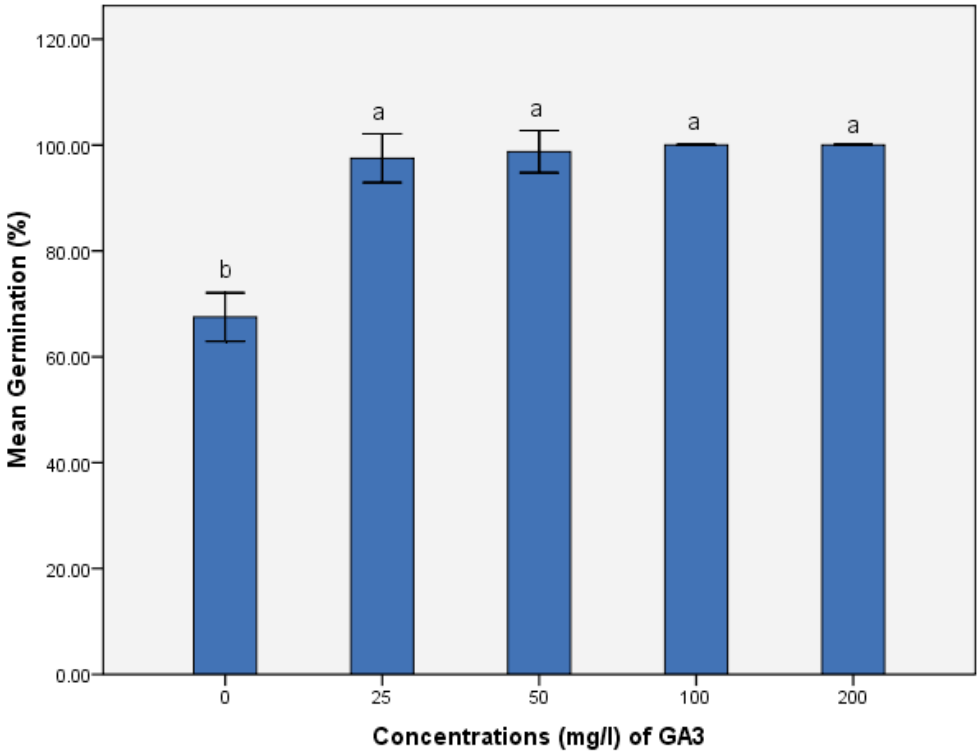

Figure 4.20. Effect of GA_3 at various concentrations on the germination percentage of okra seeds under *in vitro* condition.

Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.51. Measurement of plant height, number of branches, stem girth and number of leaves after pre-sowing treatment with GA_3 at different concentrations (mg/l) in okra plants under *in vitro* condition.

Concentrations (mg/l)	Plant height (cm)	No. of branches/plant	Stem girth (cm)	No. of leaves/plant
0	73.93±0.04e	1.75±0.50c	2.54±0.01e	18.75±0.95e
25	80.74±0.02d	1.75±0.50c	2.63±0.01d	24.00 ±0.82d
50	84.65±0.02c	2.25±0.50b	2.66±0.01c	26.75±1.26c
100	89.73 ±0.01b	2.50±0.57b	2.73±0.01b	29.25±0.96b
200	95.15±0.01a	3.00±0.00a	2.81±0.01a	34.75±1.25a

Values are means ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.52. Measurement of chlorophyll content, Fv/Fm yield and number of pods per plant after pre-sowing treatment with GA_3 at different concentrations (mg/l) in okra plants under *in vitro* condition.

Concentrations (mg/l)	Chlorophyll content (SPAD value)	Fv/Fm yield	Number of pods/plant
0	39.75±0.50e	0.702±0.01d	8.50±0.76e
25	43.21±0.01d	0.771±0.01c	16.75±1.25d
50	49.31±0.01c	0.771±0.01c	18.50±1.00c
100	54.57±0.02b	0.784±0.01b	23.00±0.86b
200	57.13±0.01a	0.796±0.01a	27.75±0.96a

Values are means ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

Table 4.53. Effect of pre-sowing treatment applied with GA_3 at different concentrations on pod characters and healthy seeds percentage of okra under *in vitro* condition.

Concentrations (mg/l)	Pod length (cm)	Pod diameter (cm)	Single pod weight (g)	Pod size (cm^2)	Healthy seeds (%)
0	4.24±0.01c	1.26±0.01c	2.75±0.02c	4.10±0.04c	86.15±0.02e
25	4.24±0.01c	1.26±0.01c	2.75±0.02c	4.10±0.08c	88.21±0.01d
50	4.24±0.02c	1.27±0.02c	2.76±0.02c	4.11±0.10c	89.45±0.02c
100	7.34±0.01b	2.04±0.02b	8.39±0.01b	14.98±0.10b	93.23±0.02b
200	7.56±0.01a	2.25±0.01a	8.66±0.01a	17.01±0.10a	97.14±0.01a

Values are mean ± standard deviation
Mean followed by same letter were not significantly different at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

4.5.4 Comparison of the effect of different concentrations of IAA; NAA and GA_3 on duration of seed germination and flowering time

4.5.4.1 Duration of seed germination

Table 4.45 showed the effect of growth regulators on duration of germination. Early germination observed with GA_3 pre-sowing treatment at 100 and 200 mg/l (4days) as compared to control (7days) while the IAA and NAA pre-sowing treatment with lower concentrations (25 and 50 mg/l) germination was delayed for 2 days when compared with control. But the higher concentrations of IAA and NAA (100 and 200 mg/l) proved to be the most inhibitory, because germination was delayed around 5 days as compared to control.

4.5.4.2 Flowering

Flowering time was influenced by pre-sowing treatment under *in vitro* condition of IAA, NAA and GA_3 at various concentrations (Table 4.46). Early flowering was obtained with GA_3 at 100 mg/l (44 days) followed by GA_3 at 200 mg/l (46 days) and IAA at 25 mg/l (46 days) in comparison with control (54 days) but there was no significant effect of IAA and NAA treatments at 100 mg/l. In this respect, flowering was attained in pre-sowing of NAA at 25 and 50 mg/l and IAA at 50 mg/l within 52 days after planting.

Table 4.54. Effect of IAA, NAA and GA_3 with different concentrations on the duration of germination of treated and control seeds of okra.

	Control	**IAA 25 mg/l**	**IAA 50 mg/l**	**IAA 100 mg/l**	**IAA 200 mg/l**
Days	7	8	8	12	0
	Control	**GA_3 25 mg/l**	**GA_3 50 mg/l**	**GA_3 100 mg/l**	**GA_3 200 mg/l**
Days	7	7	7	4	4
	Control	**NAA 25 mg/l**	**NAA 50 mg/l**	**NAA 100 mg/l**	**NAA 200 mg/l**
Days	7	8	8	12	0

0: No germination.

Table 4.55. Difference of flower opening day (blooming) in the treated and control plants under pre-sowing treatment with IAA, NAA and GA_3 at various concentrations.

	Control	**IAA 25 mg/l**	**IAA 50 mg/l**	**IAA 100 mg/l**	**IAA 200 mg/l**
Days	54	46	52	54	0
	Control	**GA_3 25 mg/l**	**GA_3 50 mg/l**	**GA_3 100 mg/l**	**GA_3 200 mg/l**
Days	54	54	54	44	46
	Control	**NAA 25 mg/l**	**NAA 50 mg/l**	**NAA 100 mg/l**	**NAA 200 mg/l**
Days	54	52	52	54	0

0: No germination.

DISCUSSION

This study compared the bioavailability of plant growth regulators at different concentrations for improving growth, yield and fruit quality when applied to okra crop by various methods (flower injection, ovary injection, stem injection and soaking). The use of the plant growth regulators (PGRs) is becoming an increasingly important aspect in agricultural and horticulture practices for many cultivated plants (Monselise, 1979). Application of plant growth regulators (PGRs) is one of the most useful tools for horticultural crops to enhance yield, improve crop quality and management (Emongor, 1997). Several reports which indicated that application of the plant growth regulators can provide germination, growth, fruit set, fresh vegetables weight and seed yields quality (Saimbhi, 1993). Growth regulators have a positive effect on fruit quality, carbohydrate, protein, vitamin contents and mineral elements content with many horticulture crops. Lemaux, (1999); Olaiya and Osonubi, (2009) informed that natural plant growth regulators or synthetic are controlled the plant activities and their productions by controlling one or more of one or more specific physiological processes within a plant. However, Gibberellic acid is safe for human health, which can be used for different aims (Iknur *et al*., 2008). Plant growth regulators play a central role in morphology and physiology of the plants. The effect of a growth regulator depends on plant species, variety, their growth stage, and concentration of chemicals that used application technique and frequency of application (Hilli *et al*., 2009).

Germination%

In pre-sowing treatments, Both 25 and 50 mg/l concentrations of IAA and NAA showed a major difference in respect of germination (98%) which meant the lower concentrations of

auxins was better than higher concentrations (100 and 200 mg/l) rather it increased the germination percentage. While GA_3 100 and 200 mg/l found most suitable because it showed highest germination percentage (100%). These results might be due to the change in the endogenous growth regulators like GA_3 leads to increase endogenous IAA level, which decreases IAA-oxidase activity (Watanabe and Stuz, 1960). In addition, Fincher, (1989) reported that by GA_3 was affected germination, and it associated enzymes, which had promotive effects.

These results in agreement with Ogbonna and Abraham, (1989) who observed that pre-sowing treatment of cowpea seeds with IAA, IBA improved seeds germination, dry matter production, flowering and yield in lower concentrations but higher concentration up to 50 ppm of IAA had the inhibitory effect on germination rate. Pre-sowing treatment with GA_3 increased rate of seed germination and plant height, but it decreased number of primary branches as compared to control. Furthermore, IAA at lower concentrations had the better effect than higher concentrations on crops, which is in line with present results. Moreover, Singh and Murthy (1987) found that pre-sowing treatment with GA at higher concentration enhanced germination while IAA and KN in low concentration in *Cassia obtusifolia.* In this respect, lower concentrations of PGRs maybe enhanced enzymatic activity that guided to the positive environment for the germination (Chauhan *et al*., 2009). Vamil *et al*., 2010 reported that GA_3, IAA, IBA, 2-4-D and NAA at 10 µM and 100µM concentrations enhanced germination percentage, seedling growth, leaf area, and chlorophyll content. Also, they decreased the germination time. In addition, reported that highest leaf area and chlorophyll content were obtained in IAA (100µM and 10µM concentrations, respectively in *Bambusa arundinaceae*. These results might be due to the change in the endogenous growth regulators

like GA_3 leads to increase endogenous IAA level which, decreased IAA-oxidase activity (Watanabe and Stuz, 1960). In addition, Fincher, (1989) reported that GA_3 was affected on germination, and it associated enzymes, which have promotive effects. Gibberellins encourage the growth of the seed to a seedling by stimulating the synthesis of digestive enzymes like amylase, which can translate the storage form of nutrients to the mobilized form so that the seed can utilize these stored nutrients to germinate.

Pod setting

Flower and ovary injection methods were applied on the okra flower using IAA, NAA and GA_3 at different concentrations. Depending on the results of this study, the percentage of pod setting per plant was higher in the lower concentrations (25 and 50 mg/l) of IAA and NAA compared to the control. Meanwhile, higher concentrations (100, 200 mg/l) of IAA and NAA treatments decreased it compared with control. Also, results indicated that GA_3 application significantly increased the pod setting of okra treated flowers as compared with control. The highest pod setting values observed with GA_3 at 25 and 50 mg/l. Application of growth regulators increased fruit set ratio as compared to control in tomato (Sasaki *et al*., 2005). Furthermore, Vijay and Jalikop, (1980) observed that application of plant growth regulators at different concentration enhanced fruit set and fruit weight of the teasel gourd. Increased pod setting percentage per plant at lower concentrations observed in this present study is in an agreement with those of El-Saeid *et al*., (2010) who obtained that IAA application at flowering stage with concentrations 25 and 50 mg/l promoted flowering while high concentrations to 100 mg/l application had an inhibiting effect in Cowpea plants. Also, Sarkar *et al*., (2002) observed GA_3 and IAA enhanced fruit setting of soybean when applied two times at 100 mg/l. However, the present results show that the significance of choosing

the suitable concentrations. Increasing of fruit setting percentage with IAA application might be due to increase of IAA endogenous, which related to decreasing the sensitivity to ethylene exerted by high level of IAA endogenous (Li-X and Meng, 1997). Moreover, IAA application increased gibberellins and cytokinins and reduced ABA endogenous level content, which this balance between their levels is important for decreasing the percentage of flower abscission of cowpea plants (El-Saeid *et al*., 2010). However, the maximum increase was recorded by concentrations 25 and 50 mg/l. In addition; Takao and Takashi (2010) informed that phytohormones are considered to the key factors in fruit setting in plants, principally auxin and gibberellins. Besides that IAA application at low concentrations stimulated growth in various crops (Bandusky and Nonhebel, 1990). On another hand, the reason may be interpreted to the physiological role of auxins in replacing the signals offered by pollination and fertilization (Nitsch, 1952; Schwabe and Mills, 1981). Furthermore, Gillaspy (1993) found that levels of auxins increased in flower after fertilization stage. In addition, the interaction between auxin and gibberellin which observed in pea and tomato for fruit growth suggesting that both work together for regulate fruit growth (Serrani *et al*., 2008; Ozga and Reinecke, 1999). The positive effect of NAA at 25 mg/l on the increase in the percentage of fruit setting in okra which agrees with the findings of Alam and Naqvi (1989) and Younus and Tigani (1977) who reported that NAA spray at the time of flowering stage prevented the flower abscission in tomato crop by enhancing auxin concentration at this important time. On the other hand, NAA application at 200 mg/l concentration showed an inhibitory effect. Grierson and Kader, (1986); Lee and Leegood, (1999), found that GA_3, IAA, IBA and NAA enhance fruit setting, ripening and reduce fruit dropping. Jules *et al*., 1981, reported that growth regulators inhibited plant growth at higher concentrations. In addition, IAA and GA_3 enhance plant growth in different concentrations (Mella *et al.,* 1997). Significant increase in

fruit size reported by Malasi (1981); Rahman *et al*., (1994); (Mathur, 1971) have reported that plant growth regulators (IAA, GA_3, respectively) produced better fruit than control in respect to length, diameter and weight. Also, Doddamani and Panchal, (1989) obtained increased fruit length and diameter of chilli fruit due to application of NAA at lower concentrations. This may be attributed to the role of GA and auxins in increasing cell division and cell elongation (Cleland, 1995; Goodwin and Mercer, 1983).

Plant height

Application of GA_3 at 25, 50 and 200 mg/l increased the plant height over control in both stem injection and pre-sowing treatments. IAA and NAA at all concentrations with stem injection produced statistically similar plant height as was produced by control treatment. In the contrary, presowing treatments with applications of IAA and NAA at 25, 50 and 100 mg/l had a significant effect on the plant height. These results might be attributed to the effects of gibberellic acid and auxins on plant height in increase cell division and cell elongation (Cleland, 1995; Ranjan *et al*., 2003). El-Otmani *et al*., (1993); Agusti *et al*., (1996) mentioned that NAA effect might be due to enhancement of cell enlargement, not cell division. GA_3 is concerned to enhance cell division and elongation (Harrington *et al*., 1996). Veer Kumar, (2002) and Sreedhar, (2003) stated that increased stem elongation might be due to stimulating action of GA_3, which alleviate the cell wall by increasing its plasticity. Auxins, after being transported to the target tissue, motivate the cells there to elongate by providing an acid medium. The acid medium could encourage the rigid cell wall to enlarge easily since the acid can weaken the inflexible structure of the cellulose, and when the cell intakes water, the cell can lengthen (Byrne, 1999). The results confirmed with those of Sarkar *et al*., (2002) and Mukhtar, (2008) who found that GA_3 and IAA applications increased the plant height of

soybean and Red sorrel, respectively. But both investigations found GA_3 at 100 ppm was efficient than IAA. Also, earlier studies reported that GA_3 increased plant height in various crops; soybean (Deotale *et al*., 1998), sesame (Sontakey *et al*., 1991), rice (Awan and Alizai, 1989) and some cowpea cultivars (Mukhtar, 2004). In addition, Sultana *et al*., (2006) observed that NAA application at 10 and 100 ppm increased the plant height of chilli.

Number of branches and leaves characters

Results clearly showed that growth regulator application increased pod size in okra. Among the concentrations of IAA, NAA and GA_3, the highest pod size was found with 25, 50 mg/l of IAA and NAA while the lowest pod size around was recorded in 25 and 50 mg/l of GA_3 and control. With GA_3 at 100 and 200 mg/l concentrations, there was a significant difference in the pod in comparison with control. Chhipa and Lal, (1988) found that IAA application increased number of branches per plant in wheat plant. GA_3 increased number of branches per plant was reported earlier in soybean (Deotale *et al*., 1998); and sesame (Sontakey *et al*., 1991). IAA application increased leaf number per plant in onion (Abdul Hye *et al*., 2002) and GA_3 increased leaf number per plant in bell pepper (Abdul *et al*., 1988); (Shishido and Saito, 1984). Mukhtar, (2008) found that GA_3, and IAA treatment at 100 ppm increased leaf number and leaf area and chlorophyll content in Hibiscus sabdariffa L. Moreover, Salah *et al*., (1989) mentioned a significant increase in the leaf length in onion by application of GA_3. Similar reports have been obtained by (Vamil *et al*., 2010) who observed IAA application increased the leaf area of plants and chlorophyll content. This may be attributed that GA_3 and IAA increase the division and elongation of the cells led to better vegetative growth of plants. Furthermore, Wanyama, (2006) informed that GA_3 application increases branches number by breaking apical dominance. Jordi *et al*., (1995) informed that GA_3 delays the loss of

chlorophyll. In this respect, NAA at 30 ppm, mepiquat at 120 ppm and their mixture enhanced leaf area index, leaf chlorophyll content, photosynthetic rate and seed yield of blackgram (Kannan *et al*., 2003). Also, NAA at 10 ppm enhanced branch number/plant in chilli (Sultana, 2006) and yard long bean at 15 ppm (Resmi and Gopalakrishnan, 2004). Also, Resmi and Gopalakrishnan, (2004) found that NAA application delayed flowering by 8 days at 45 ppm and 4 days at 15 ppm.

Yield

All growth regulators (IAA, NAA and GA_3) enhanced pod yield per plant of okra in this study dependent on their concentrations. The data indicated that the IAA and NAA at 25, 50 and mg/l increased pod yield more efficiently than the control and other concentrations. However, GA_3 at 100 and 200 mg/l had a 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. Furthermore, another important factor is numbered of branches/plant, which offered a chance to the plant to carry more flowers therefore, higher pods. 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 who lead naturally to increase fruit size by increasing the permeability of a fruit cell wall (Abduljabbar *et al*., 2007). IAA and GA_3 application at 100 ppm increased the yield of rice and soybean (Awan and Alizia, 1989; Reena *et al*., 1999) respectively. Furthermore, NAA at 15 ppm after 15, 30 and 45 days of sowing increased fruit set and productivity of the yard long bean

(*Vignaunguiculata* var. *sesquipedalis* (L.). The positive effect of NAA on production might be due to enhancement of vegetative growth, reduced flower dropping and fruit abortion (Resmi and Gopalakrishnan, 2004).

Seeds yield

Significant in percentage of healthy seeds were observed after IAA, NAA and GA_3 treatments at all concentrations compared with control but 100 mg/l of IAA and NAA treatments, which had minimum healthy seeds. The highest concentration of IAA (200 mg/l) with flower injection and 100 mg/l with ovary injection methods inhibited seed production and produced 100% of Aborted seeds (Stenospermocarpy). A significant increase in seed abortion was obtained after IAA and NAA treatment at 100 mg/l. On the contrary, a significant decreased of seed abortion percentage was observed after GA_3 treatment at 200 mg/l compared with control. The increase in seed yield due to GA_3 application and other treatments may be related to improving vegetative growth (leaf area and leaf number plant. The present observations were more or less in confirmation with Sarkar *et al*., (2002) who obtained that GA_3 application at 100 ppm to soybean produced the highest yield of seeds per plant followed by 100 ppm of IAA, and the lowest yield obtained with 200 ppm of IAA. The data showed that the lower concentrations of IAA and NAA (25 and 50 mg/l) increased seed yield more efficiently than the higher concentrations (100 and 200 mg/l). In addition, Awan and Alizai, (1989); Kumer *et al*., (1996) observed that GA_3 at 100 ppm increased seeds yield in rice and okra. There some hypothesis explained the role of auxin in causing fruit development. First, it is suggesting that pollen and pollen tubes have a growth stimulator, maybe auxin, which is responsible for fruit development; mostly, by enhancing growth of the ovary while another one suggesting that developing seeds have auxin that causes enlargement

of the fruit (Dean, 1978). The results are in conformity with the results of Elassar *et al*., (1974) who found artificial auxin induced parthenocarpic fruits when applied directly to flower (at anthesis) in muskmelon. Also, Kim *et al*., 1992 observed that applied IAA and NAA directly to the ovary were activated fruit set and following growth of *Cucumis sativus* by increasing the auxin concentration in it, which is responsible for fruit set.

Pods nutritional contents

GA_3 concentrations up to 100 mg/l, IAA and NAA at 25 and 50 mg/l increased TSS content of pods, vitamin C content K content. On the contrary, application with GA_3, IAA and NAA with all concentrations (25, 50, 100 and 200 mg/l) had significantly reduced pod contents of Ca and Mg and as compared to control. Maximum Ca and Mg content in the pod was recorded in control followed by 25 mg/l and 50 mg/l but GA_3 applications increased Na content per pod, which was high with 100 mg/l treatment followed by 200 mg/l and the lowest content was observed with control treatment. Iron (Fe) content was increased significantly by the application of IAA and NAA with all concentrations compared to control.

The increase of vitamin C content due to GA_3 application could be due to the enhancement of ascorbic acid biosynthesis or to the synthesized of ascorbic acid from oxidation through ascorbic acid oxidase (Ouzounidou *et al*., 2010). All growth regulators at all concentrations have been found affected the biochemical parameters of okra fruit. Looney, (1998) mentioned that growth regulators have an important effect on plant physiological processes. Also, Nickel, (1978) and Grierson and Kader, (1986) reported that growth regulators influence physiological and biochemical processes of plants such as flowering, fruit set and regulation

of chemical composition of plants. Olaiya and Adigun (2010) obtained in tomato that the values of soluble solid content increased with NAA and IAA applications compared to control. In addition, Ouzounidou *et al*., (2010); found that GA_3 application increased soluble solids and ascorbic acid in green peppers. In addition, GA_3 treatment increased fruit content of ascorbic acid in chilli better than other growth regulators (Chaudhary *et al*., 2006). GA_3 and IAA and coucnt milk applications increased fruit content vitamin C, A and B6 in *Abelmoschus esculentus* and *Solanum gilo*. In addition, Mukhtar, 2008, obtained that application of GA_3 at 100 mg/l to increased carbohydrate and vitamins A, B6, C, P, K, Na, Cu and Zn contents of *H. sabdariffa* leaves while it decreased Ca, mg, Mn and Fe. Moreover, IAA application at 100 mg/l reduced Ca, mg, Na, Cu and Zn leaf content, but it increased P, K, Mn and Fe.

CHAPTER 5

SUMMARY AND RECOMMENDATION

5.1 FLOWER INJECTION METHOD APPLICATION OF PLANT GROWTH REGULATOR (IAA, NAA AND GA_3) AT DIFFERENT CONCENTRATIONS

5.1.1. Flower injection with IAA

Injection method was applied on the okra flower using IAA at different concentrations. The percentage of pod setting per plant was higher in 25 and 50 mg/l concentrations of IAA compared to the control. IAA flower injection application with different concentrations had significant differences in pod length compared with control. The highest pod length was observed with flowers injected with 25 mg/l while lowest pod length was recorded in the control pod. The highest mean value of pod diameter was obtained for 25 mg/l IAA concentration. Flower injection application of IAA increased the weight of pods. In this respect, the effect was dependent on the level of IAA concentration. In the present study, higher healthy seed percentage per plant was recorded at flower injection treatment with 25 mg/l concentration followed by 50 mg/l treatment and control. The highest dose of IAA (200 mg/l) in this investigation inhibited seed production and produced 100% of Aborted seeds. (Stenospermocarpy) Even as the lowest dose (25 mg/l) was the best treatment for healthy seed production followed by control. Total soluble solids (TSS) were markedly increased by IAA application at all different concentrations. Okra pod showed the highest TSS with 25 mg/l concentration of IAA. The vitamin C in okra pods was significantly increased by IAA flower injection treatment. Vitamin C pod content was increased over the control at 25, 50 and 100 mg/l. IAA at 25 mg/l resulted in the highest pod content of vitamin C while flower injection treatment with 200 mg/l had the least effect on vitamin C content. The highest K

content was obtained in 25 mg/l of IAA concentration. Meanwhile, the control produced the lowest K content. In addition, iron (Fe) content increased significantly by the application of IAA with all concentrations compared to control.

5.1.2. Flower injection with NAA

NAA at 50 and 100 mg/l concentrations had a lower pod setting percentage compared to 25 and 0 mg/l. But NAA at 200 mg/l concentration inhibited the pod setting (0%). Pod length was significantly increased with 25 mg/l followed by 50 mg/l. The single pod weight increased in 25, 50 and 100 mg/l. Healthy seed percentage was higher in NAA 25 and 50 mg/l treated flowers whereas NAA 100 mg/l treated flowers exhibited lower healthy seed percent than control. In this respect, percentage of aborted seeds was highest in NAA 100 mg/l. NAA at 25 mg/l treatment had the lowest percentage of aborted seeds compared to other treatments. All of the NAA concentrations (25, 50 and 100 mg/l) caused a significant increase of TSS content compared with control. The maximum content of TSS per pod was achieved by 25 and 50 mg/l of NAA. Both concentrations of NAA (25 and 50 mg/l) increased significantly vitamin C content per pod. Maximum vitamin C content was recorded in the pod harvested from 25 mg/l NAA treated flowers and found in descending order of 50 mg/l, control (0 mg/l) and 100 mg/l. Likewise, potassium (K) content per pod was also found significantly highest among all concentrations. Minimum potassium content in the pod was recorded in 100 mg/l and control pod.

5.1.3 Flower injection with GA_3

The highest pod setting (100%) had been obtained in case of GA_3 at 100 mg/l, which significantly differed from control. Maximum pod length (10.58 and 10.59 cm) was observed

by applying GA_3 of 50 and 100 mg/l while minimum length was obtained in control. GA_3 at 25 and 200 mg/l increased the pod length over control (6.90 and 8.30 cm), respectively. The heaviest pods obtained with GA_3 at 50 and 100 mg/l as compared to 200 and 25 mg/l applications. All the concentrations had been increasing trend of healthy seed production compared with control. GA_3 at 50, 100 and 200 mg/l had higher healthy seeds per pod than 25 mg/l and control. Minimum healthy seeds were obtained in control treatment. In another hand, maximum TSS was observed in 100 mg/l treatment, which differed significantly from all other treatments. GA_3 concentrations upto 200 mg/l increased TSS content of pods. Vitamin C content was appreciably affected by GA_3 at 100 mg/l. Maximum K content was recorded in the application of 100 mg/l. On the contrary; flower injection application with GA_3 had significantly reduced pod contents of Ca; Mg and Fe as compared to control. Maximum Ca, Mg and Fe content in the pod was recorded in control followed by 25 mg/l and 50 mg/l. The highest Na content per pod was obtained by 100 mg/l treatment followed by 200 mg/l and the lowest content was observed with control treatment.

5.2. OVARY INJECTION METHOD APPLICATION OF PLANT GROWTH REGULATORS AT DIFFERENT CONCENTRATIONS

5.2.1. Ovary injection method by using Indole acetic acid (IAA)

The Percentage of pod setting was higher at 25 mg/l and lower at 100 mg/l as compared to control. Accordingly, the highest pod length was found in 25 mg/l of IAA concentration. IAA at 25 and 50 mg/l led to increase the pod diameter by 6-20%. However, there was a significantly decrease in the pod characters with the increase in concentration. Pod setting percentage of the ovary treated by the application at 200 mg/l IAA concentration was drastically reduced resulting in very few pods were set in the plants. Furthermore, maximum TSS was observed in 25 mg/l IAA treatment. IAA concentration up to 100 mg/l decreased

TSS content of pods. IAA at lower concentrations (25 and 50 mg/l) produced significantly more vitamin C content of pods than control. The K, Mg, Fe and Na contents in the pod were significantly affected by IAA treatments at different concentration's highest contents of K, and Fe were noted for IAA at 25 mg/l concentration. In particular, all concentrations of IAA showed decreased pods content with Mg per pod in comparison with control.

5.2.2 Ovary injection method by using Indole acetic acid (NAA)

The higher percentage of pod set (24%) occurred at lower concentration (25 mg/l) over the control treatment. The TSS content and vitamin C in the pod were significantly influenced by the different concentrations of NAA compared with the control pod. NAA at 25 and 50 mg/l ovary treatments significantly increased the average of pod length compared to control pods. NAA at lower concentrations (25 and 50 mg/l) had a better effect on pod diameter, pod size and single pod weight than control. The highest healthy seed percentage per pod was recorded at 25 mg/l of NAA concentration while the highest aborted seed percentage was observed at 100 mg/l. The potassium and iron contents increased with NAA applications at 25 and 50 mg/l whereas, Ca, Mg and Na decreased with same applications as compared to control.

5.2.3 Ovary injection method by using gibberellic acid (GA_3)

Maximum pod setting occurred in 25 and 50 mg/l GA_3 whereas minimum was obtained in GA_3 at 200 mg/l and control. GA_3 (25 and 50 mg/l) produced bigger pod size with heavier pod weight than other concentrations. The biggest and heaviest pods were found with 50 mg/l whereas the smallest and lightest pods were produced by control treatment. The percentage of healthy seeds was highest in 50 mg/l GA_3 concentration. 200 mg/l application had lower

healthy seeds percentage (70.80%) compared to other treatments. Significant differences among treatments were observed in the case of seed abortion intensity which was maximum in 200 mg/l treatment and minimum in 50 mg/l. The highest TSS was observed in 50 mg/l compared with control. It was observed that all concentrations of GA_3 increased the vitamin C in pod. Potassium contents of pods are generally higher in different concentrations of GA_3 having comparison with control. The results also showed that the calcium and iron significantly reduced in different concentrations as a whole compared with control. In addition, all the treatments had decreasing trend towards levels of magnesium (Mg) with control, except 25 mg/l treatment which had maximum content of Mg. GA_3 treatment had increased sodium (Na) content in pods compared with control.

5.3. STEM INJECTION METHOD APPLICATION OF PLANT GROWTH REGULATORS AT DIFFERENT CONCENTRATIONS

5.3.1. Stem injection method by using Indole acetic acid (IAA)

Plant height, number of branches, stem girth and number of leaves per plant were significantly affected by different concentrations of IAA applied by stem injection method. The data revealed that IAA at 100 and 200 mg/l produced the taller plants than at 25, 50 mg/l and control treatments respectively. IAA at 25 and 50 mg/l had significantly higher branch number than control. However, 200 mg/l applications had the highest branch number than other treatments. IAA applications at all concentrations (25, 50, 100 and 200 mg/l) were more effective in stem elongation than control. Chlorophyll content per pod did not increase with an increase of IAA concentrations. Similarly, Fv/Fm yield was not affected by the different application levels of IAA in control and 200 mg/l. Pod yields per plant increased with an increase of IAA concentrations as compared to control. Pod length and pod diameter

did not increase with an increase of IAA concentrations. Likewise, healthy and aborted seed percentages did not influence by the different concentrations of IAA applied.

5.3.2. Stem injection method by using Naphthalene acetic acid (NAA)

The tallest plant was observed with lower concentration (25 mg/l) as compared to other treatments. The data revealed that, 25 mg/l of NAA produced the maximum number of branches as compared to control. Stem girth significantly increased with an increase in the level of NAA except 200 mg/l. The chlorophyll content per leaf did not influence by the different concentrations of NAA. There was also found with the minimum quantum yield of primary photochemistry (Fv/Fm) in 200 mg/l. There was no significant difference in the pod length and pod diameter produced from plant treated with NAA at various concentrations. Also, there was no difference between concentrations of NAA in pod size and individual pod weight as compared to control. It was observed that the effect of NAA at different concentrations injected to stem was not affected on healthy and aborted seed percentages. However, NAA had no effect on pod contributing and seed production by stem injection method.

5.3.3 Stem injection method by using Gibberellic acid (GA_3)

The higher concentrations (100 mg/l) of GA_3 greatly increased the plant height compared to the control. The lower concentrations had lesser plant height, but it was higher than the control. The treated plants generated higher number of branches over control. Among the GA_3 application, 100 mg/l of GA_3 induced maximum number of branches followed by 200 mg/l as compared to control. In the contrary, GA_3 application at 100 mg/l induced the highest value of stem girth over the control. The highest number of leaves per plant was obtained by 100 mg/l, and the lowest leave number/plant was observed with control treatment. The

chlorophyll content in leaves was affected significantly by different concentrations of GA_3. It was found that all concentrations of GA_3 (25, 50, 100 and 200 mg/l) increased chlorophyll content per leaf by 37, 45, 60 and 55% of the control. The total pod per plant, pod length, pod diameter, pod size, single pod weight and healthy seeds percentage per pod were significantly affected by different concentrations of GA_3. Among the concentration's treatments, 100 mg/l had the maximum number of pod per plant in comparison with control. Pod diameter was found the maximum with 100 mg/l. Significantly highest pod size was obtained in 100 mg/l followed by 200 mg/l. In this respect; pod weight recorded significantly highest in 100 mg/l. Pod harvested from 25 mg/l treated plants had significant highest aborted seeds percentage followed by 50 mg/l. 100 and 200 mg/l treatments had increased the production of healthy seeds compared with control. 100 mg/l treatment had maximum healthy seeds per pod followed by 200 mg/l. However, 100 mg/l application produced the longest pods.

5.4 SEED- PRETREATMENT METHODS

5.4.1. Seed was soaked in indole acetic acid (IAA) and grown in field (*In vivo*)

IAA at the concentration of 25 mg/l had a remarkable effect on germination percentage compared to control. Whereas, IAA at 50, 100 and 200 mg/l concentrations significantly reduced germination percentage as compared with control. Plant height at the end of harvesting stage, was higher in IAA 25 mg/l treated plants than control plants. Number of branches per plant was maximal in plant in IAA at 25 mg/l treatment. Stem girth was highest in 25 mg/l IAA treated plants. The higher chlorophyll content (represented by SPAD value) was obtained under treated seeds with 25; 50 and 100 mg /l compared to control. The lowest chlorophyll content was found by 200 mg/l pre-sowing treatment. Likewise, the maximum quantum yield of primary photochemistry (Fv/Fm) was significantly increased with

application of 25, 50 and 100 mg/l compared to the control. However, 200 mg/l pre-sowing seed treatment decreased Fv/Fm 14% in comparison with control. The highest number of pods per plant (23.50) was produced by the pre-sowing seed treatment with 25 mg/l, and the lowest (8.50) was observed by control plants. Pod length was significantly increased by the application of IAA at 25, 50 and 100 mg/l compared to control. The highest pod diameter and weight were obtained in 25 mg/l IAA pre-sowing treatment. The heaviest pods (10.52 g) were observed with 25 mg/l pre-sowing treatment. The Maximum healthy seed percentage was observed in 25 mg/l while the minimum was obtained in 200 mg/l. The highest TSS and vitamin C were recorded in 25 mg/l IAA concentration compared to control. Potassium content per pod was higher in IAA at 25, 50 and 100 mg/l than control and 200 mg/l treatments. The iron content per pod was higher in all IAA pre-sowing treatments than 200 mg/l and control.

5.4.2. Seed was soaked in Naphthalene acetic acid (NAA) and grown in field (*In vivo*)

The highest germination percentage (100%) was observed in NAA 25 mg/l followed by NAA 50 mg/l (83%) in comparison with control (77.5%) whereas, the lowest germination percentage was observed in NAA 200 mg/l (42%). It was obtained that 25 and 50 mg/l NAA concentrations exhibited a highly significant effects on the plant height compared with control. The maximum number of branches per plant was observed with 50 mg/l application of NAA, and the minimum was recorded in 200 mg/l. The highest chlorophyll content and quantum yield of primary photochemistry (Fv/Fm) was found in 50 and 25 mg/l, respectively. Pre-sowing treatment with NAA at 50 mg/l had the highest number of pods per plant. Similarly, 50 mg/l pre-sowing treatment gave the highest pod length while 200 mg/l produced the shortest pod. In this respect, the largest pod diameter was obtained with 50 and 25 mg/l

application, respectively. Pre-sowing treatment with 50 mg/l gave the greatest pod weight per plant. In addition to that better yield was found in the 25 and 50 mg/l pre-sowing treatment compared to control. NAA at 25, 0, 50, 100 mg/l concentrations had significantly higher healthy seed percentage than control and 200 mg/l. Total soluble solids, vitamin C and potassium were affected significantly by different concentrations of NAA. The maximal TSS was found in 50 mg/l as compared with 0, 25, 100 and 200 mg/l. The highest vitamin C and K content per pod was found with the treatment of 50 mg/l. While Iron was increased under NAA pre-sowing treatment as compared to control.

5.4.3. Seed was soaked in Gibberellic acid (GA_3) and grown in field (*In vivo*)

The observations of the experiment showed that GA_3 25 and 50 mg/l resulted in equal 97.5 percent germination which followed by 100 and 200 mg/l while control treatment showed the lowest germination. Plant height of okra plant was significantly influenced by the application of GA_3. The highest plant height was recorded from the highest dose of GA_3 (200 mg/l). Application of higher doses of GA_3 increased the stem girth, and the highest was with 100 and 200 mg/l. The highest chlorophyll content and quantum yield of primary photochemistry (Fv/Fm) was found in 200 mg/l. Pod length and pod diameter were also affected with the GA_3 treatments. Highest pod length was recorded at 200 mg/l, and the lowest was at control treatment. Similarly, the highest value of diameter was recorded at 200 mg/l. In this context, 200 mg/l pre-sowing treatment produced the heaviest pods. Gibberellic acid pre-sowing treatment of okra seeds significantly increased healthy seed percentage compared to control pre-sowing treatment. The increase in healthy seeds percentage per fruit was linear with increasing GA_3 concentration.

Seed soaking with GA_3 had a significant effect on total soluble solids, vitamin C and potassium in pods. In the GA_3 pre-sowing treatment, the relative increase in Total soluble solids (TSS) was significantly more in higher concentrations (100 and 200 mg/l) than control. The vitamin C in pods showed a remarkable increase with applied GA_3 at 100 and 200 mg/l. The highest potassium content was observed with 200 mg/l treatment compared to other treatments. Maximum magnesium content of pod was recorded in the control pod. GA_3 had a positive effect on Na content of pod. The higher concentrations increased Na content significantly in the pod as compared to control.

Early germination was observed with GA_3 pre-sowing treatment at 100 and 200 mg/l (5 days) as compared to control (7 days) while the IAA and NAA pre-sowing treatment with lower concentrations (25 and 50 mg/l) germination was delayed for 2 days when compared with control. However, the higher concentrations of IAA and NAA (100 and 200 mg/l) proved to be the most inhibitory, because germination was delayed for 6 days as compared to control. Flowering time was influenced by pre-sowing treatment of IAA, NAA and GA_3 at various concentrations. Early flowering was obtained with GA_3 at 100 mg/l (42 days) followed by GA_3 at 200 (46 days) and IAA at 25 and 50 mg/l in comparison with control but there was no significant effect of GA_3 treatments at 25 and 50 mg/l. In this respect, IAA and NAA applications at higher concentrations (100 and 200 mg/l) delayed the onset of flowering by more than 3 days.

5.5. Seeds WERE SOAKED AND GROWN IN MS (MURASHIGE AND SKOOG'S) MEDIUM (*IN VITRO*)

5.5.1. Seeds were soaked in indole acetic acid (IAA) (*in vitro*)

The germination percentage was higher in 25 and 50 mg/l IAA treated plant compared to control (0 conc.). However, treatment with IAA at 200 mg/l concentration inhibited the seed germination (0%). The highest plant height was observed in 50 mg/l. The maximum number of branches was found in 50 mg/l whereas the lowest branches were observed in control treatment. The number of leaves was observed higher in 50 mg/l IAA than other all concentrations and control. The chlorophyll content, Fv/Fm yield and number of pods per plant were higher in 50 mg/l treated concentration. Chlorophyll content was higher in all IAA concentrations compared with control (0 mg/l) except 200 mg/l. Pre-sowing treatment with 50 mg/l concentration produced the highest number of pods (20.75) as compared to other treatments. The highest pod length was observed in 50 mg/l, and the minimum was found in control (0 mg/l). The biggest pod size was found in 50 mg/l while control treatment gave the smallest pod size. Sowing treatment with IAA produced the highest aborted percentage was observed in 100 mg/l, and the lowest aborted seed percentage was found in control and 25 mg/l.

5.5.1. Seeds were soaked in naphthalene acetic acid (NAA) (*In vitro*)

The maximum germination percentage (100%) was observed in NAA 25 mg/l whereas, the minimum germination percentage was observed under NAA 100 mg/l. But treatment with NAA at 200 mg/l concentration inhibited the seed germination (0%). Plant height was exhibited higher in 25 mg/l level of NAA. Number of branches per plant varied significantly with variation in NAA concentrations. The number of branches per plant increased with rate

of 25 and 50 mg/l application, and the higher dose of NAA (100 mg/l) had fewer numbers of branches per plant than other treatments numbers of branches per plant, but it was same to the control. Effect of NAA application was found to be significantly on stem girth of plants. The chlorophyll content and the maximum quantum yield of primary photochemistry (Fv/Fm) differ significantly with each other due to the effect of different doses of naphthalene acetic acid (NAA). The maximum number of leaves, chlorophyll content and highest value of Fv/Fm were noted under 25 mg/l. NAA at 25, 50 and 100 mg/l under *in vitro* condition had the highest number of pods per plant, whereas control treatment (0 mg/l) had the lowest number of pods. In this respect, 25 mg/l pre-sowing treatment gave the highest pod length in comparison to control treatment. The largest pod diameter was obtained with 25 mg/l application followed by 50 and 100 mg/l as compared to control. Pre-sowing treatment with 25 mg/l also gave the greatest value of the pod weight per plant. The higher healthy seed percentage was found in 25 mg/l. In another word, 54.9% aborted seeds were recorded in 100 mg/l pre-sowing treatment.

5.5.3. Seeds were soaked in gibberellic acid (GA_3) (*In vitro*)

The germination percentage (100%) was observed higher in GA_3 at 100 and 200 mg/l than GA_3 25 and 50 mg/l and control treatment. GA_3 application at different concentrations under *in virto* condition significantly increased the plant height, branches number, stem girth and number of leaves per plant. Gibberellic acid (GA_3) treatments significantly increased the stem girth of okra compared to the control during study. Maximum increase in stem girth was obtained at concentration of 200 mg/l GA_3 treatment of okra. The chlorophyll content, Fv/Fm yield and number of pods per plant were higher in 200 mg/l treated concentration followed by 100, 50 and 25 mg/l concentrations respectively. Furthermore, the maximum pod length was

observed in 200 mg/l, and the minimum was found in 50, 25 mg/l and control. Soaked seeds under *in vitro* conditions with 200 mg/l concentration of GA_3 gave the biggest pod size while 0, 25 and 50 mg/l treatments gave the smallest pod size. The 86.15% healthy seed were found in 0 mg/l GA_3 while 97.14 % healthy seeds were recorded in 200 mg/l. Early germination was observed with GA_3 pre-sowing treatment at 100 and 200 mg/l as compared to control while the IAA and NAA pre-sowing treatment with lower concentrations (25 and 50 mg/l) germination was delayed for 2 days when compared with control. However, the higher concentrations of IAA and NAA (100 and 200 mg/l) proved to be the most inhibitory, because germination was delayed around 5 days as compared to control. In addition, flowering time was influenced by pre-sowing treatment under *in vitro* conditions of IAA, NAA and GA_3 at various concentrations. Early flowering was obtained with GA_3 at 100 mg/l followed by GA_3 at 200 mg/l, IAA at 25 and GA_3 at 200 mg/l in comparison with control but there was no significant effect of IAA and NAA treatments at 100 mg/l.

From the above discussion, it can be concluded that 25 mg/l of IAA, NAA and 100 and 200 mg/l of GA_3 concentrations were the best for okra growth and development. Finally, it can be summarized that innovative flower injection and ovary injection methods were better than stem injection; seed soaked and pre-sowing *in vitro* techniques. So it can be recommended that all these techniques can be used commercially in the vegetable industry. The internal application of flower, ovary and stem injections can reduce the chemical and production cost without hazardous any environmental pollution.