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# EVALUATE THE EFFECTS OF GIBBERELLIC ACID AND SOME NATURAL EXTRACTS ON THE MORPHOLOGICAL FEATURES AND ANATOMICAL STRUCTURE OF *FICUS BENJAMINA* L. PLANTS

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## Abstract

To evaluate the effects of the individual foliar application with gibberellic acid ($GA_3$) or with the natural extracts; active dry yeast, *Moringa oleifera* and *Aloe vera* and the combination between them on the morphological characters of *Ficus benjamina* L. plants and the anatomical structure of leaves. The results revealed that treating *F. benjamina* plants separately either with $GA_3$ at 200 ppm or with yeast extract at 8g/l gave the highest significant values of plant height, number of leaves/plant, root length and fresh weights of leaves, stems and roots, in both seasons. The exception was in the branches formation; hence it was increased with $GA_3$ at 150 ppm and the same dose of yeast extract (8g/l). The lowest values of the above mentioned characters were noticed either separately with $GA_3$ at 50 ppm or with *Aloe vera* extract at 100 ml/l. The combination between $GA_3$ at 200 ppm and yeast extract at 8g/l gave the highest significant values of most the morphological characters and the anatomical structures (Thickness of; midvein, lamina, palisade tissue and spongy tissue, in addition to main vascular bundle of midvein dimension and the mean vessel diameter). While treated the plants with $GA_3$ at 50 ppm combined with *Aloe vera* extract at 100 ml/lgave the lowest values of plant height, number of leaves/plant and fresh weight of leaves, stems and roots.

***Key words:*** *Ficus benjamina*, Gibberellic acid ($GA_3$), Yeast extracts, *Moringa oleifera* extracts, *Aloe vera* extracts, Anatomical structures.

## Introduction

*Ficus benjamina* is a medium size tree (30 m tall) with elegant glossy green leaves, graceful arching branches and smooth, light gray bark with several spreading branches from the base commonly known as weeping fig. *F. benjamina* is native to tropics South Africa, Australia, tropical central Africa and West Africa, and belongs to family Moraceae (Lansky *et al*., 2008). *F. benjamina* L. is a popular tree worldwide cultivated for ornamental purposes in temperate areas, due to its elegant growth and tolerance of poor growing conditions. It requires a moderate amount of watering in summer to keep it from drying out in the winter. For landscape purposes, creative growers have added even more interest to this already beautiful plant with several unusual techniques. The trunks of young fig trees are very flexible and it could binding together the stems of three small seedlings, tying an open knot in the trunk, or winding the stem of a young tree. It has a medicinal effect and well known due to its medicinalpotential, indigenous communities employ this plant to treat skin disorder, inflammation, malaria, vomiting and it's also used as antimicrobial, antipyretic and antinociceptive (Almahyl *et al*., 2003). The leaves, barks and fruits containvarious bioactive constituents like cinnamic acids, lactose, naringenin, quercetin, caffeic acid and stigmasterol (Sirisha *et al*., 2010).

Chemical growth regulators especially gibberellins enhance plant growth and internode length by increasing the cell division and enlargement. It has been reported that $GA_3$ can increase the stem length, stem diameter, number of branches, root length, as well as fresh and dry weights in many woody species (Mohamed, 2011 and Ibrahim *et al*., 2010).

The use of extracts of certain plants as biostimulants activators for plants such as yeast in improving the growth of agriculture crops especially ornamental plants is highly recommended as an environment friendly and safe

approach to get better plants without being forced to use chemical nutrients or synthetic growth regulators that may harm the environment. It has been indicated that yeast extracts could enhance the root and shoot growth (Abdel-Latif, 2006 and Ahmed, 2002) and chemical compositions which could increase the N, P and K contents (Ahmed, 2002; Desouky, 2004; Abass, 2008 and Emam 2010).

Mvumi *et al*. (2013) stated that *Moringa oleifera* leaf extract considered an organic extract which increases vegetative growth, cheap and environmentally safe; the extract contains the most common form of naturally occurring cytokinin in plants called zeatin. It performs better when used in conjunction with synthetic fertilizers (Balakumbahan and Rajamani, 2010 and Muhamman *et al*., 2013). In addition to zeatin, the *Moringa* extracts is also rich in ascorbates, phenolic compounds, K and Ca (Makkar *et al*., 2007).

Finally, the aqueous leaf extract of *Aloe vera* could be useful as a natural plant growth regulator (Dongzhi *et al*., 2004). It could induce the root formation in plant tissue culture. Moreover, the shoot and root growth can be increased by *Aloe* extracts in many ornamental species (Padmaja *et al*., 2007 and Hanafy *et al*., 2012).

The present study aimed to evaluate the effects of gibberellic acid and some natural extracts on the morphological features and anatomical structure of *F. benjamina* L. plants.

## Materials and methods

This study was carried out at the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, during the two seasons; 2014/2015 and 2015/2016. The aim of this study was to evaluate the effects of the individual foliar application with gibberellic acid ($GA_3$) or the natural extracts; active dry yeast, *Moringa oleifera* and *Aloe vera* and the combination between them on the vegetative growth and the anatomical structure of leaf of *F. benjamina* L. plants.

### Experimental Procedures

*Ficus benjamina* saplings, 15-20 cm long, were individually planted in 30 cm diameter pots filled with a mixture of peat moss and sand at 1:1 (v:v). Plants were sprayed after one month form planting with $GA_3$ at concentrations 0, 50, 100, 150, and 200 ppm and with the natural extracts; active dry yeast at 4 and 8 g/l; *Moringa oleifera* at 5 and 10 ml/l and *Aloe vera* at 100 and 150ml/l, in addition to control plants. $GA_3$ was applied either individually or in combination with each concentration of active dry yeast, *Moringa oleifera* and *Aloe vera* extracts. The spraying was repeated every 15 days in both seasons. The NPK recommended dose; 3 g/pot was applied monthly throughout the growing seasons.

The physical and chemical characteristics of the medium used for growing *F. benjamina* plants in 2014/2015 and 2015/2016 seasons are shown in Table (1). The soil chemical analysis was conducted using the methods described by Jackson (1973).

### Preparation of natural extracts

#### Preparation of active dry yeast extract (ADY)

Powder of active dry yeast (4 and 8 g) were put separately in two glass beakers containing 100 ml water and 5g sugar, then kept in a dark warm place for 30 min. The contents of the beakers were filtered, then collaborated by water to aliter volume and left overnight before spraying on the plants (Skoog and Miller, 1957). Chemical compositions of the active dry yeast used are shown in tables 2a & b.

#### Preparation of *Moringa oleifera* leaves extract (MLE)

The *Moringa oleifera* leaves were washed with distilled water and dried under room temperature for three

**Table 1:** Physical and chemical characteristics of the medium used for growing *Ficus benjamina* plants.

| Parameter | Average | Parameter | Average |
|---|---|---|---|
| Medium | Sand and peat moss | Soluble Anions (meq/l) | |
| pH | 7.20 | $HCO_3^-$ | 0.35 |
| EC (mmhos/cm) | 0.92 | $Cl^-$ | 1.06 |
| Soluble Cations (meq/l) | | $SO_4^{--}$ | 3.20 |
| $Ca^{++}$ | 2.31 | Available N (ppm) | 58.00 |
| $Mg^{++}$ | 0.31 (ppm) | Available K | 430.00 |
| $Na^+$ | 1.76 | | |
| $K^+$ | 0.23 | | |

**Table 2a:** Chemical composition of the active dry yeast.

| Components | Concentration | Components | Conc. µ/g |
|---|---|---|---|
| Proteins | 47.0% | Niacin | 300-500 |
| Carbohydrates | 33.0% | Pyrodoxin | 28.00 |
| Minerals | 8.00% | Pantathenate | 70.00 |
| Nucleic acids | 8.00% | Piotin | 1.300 |
| Lipids | 4.00% | Cholin | 4000 |
| Thiamine | 60-100µ/g | Folic acid | 5.13 |
| Riboflavine | 35-50µ/g | VitB12 | 0.00 |

**Table 2b:** Chemical composition of the active dry yeast.

| Minerals | Dose conc. (mg/g) | Minerals | conc. (mg/g) |
|---|---|---|---|
| Na | .120 | Cu | .800 |
| Ca | .750 | Se | 0.10 |
| Fe | 0.02 | Mn | 0.02 |
| Mg | 1.65 | Cr | 2.20 |
| K | 21.0 | Ni | 3.00 |
| P | 13.5 | Va | 0.04 |
| S | 3.90 | Mo | 0.40 |
| Zn | 0.17 | Sn | 3.00 |
| Si | 0.03 | Li | 0.17 |

weeks, pulverized in to coarse form (1000 g) with acrestor high speed milling machine. The coarse form was macerated in absolute ethanol, and then left for 48h. After that the extract was filtered through muslin cloth on a plug of glass wool in a glass column. The obtained ethanol extract was concentrated and evaporated to dryness using rotary evaporator at an optimum temperature between 40 and 45°C to avoid denaturation of the active ingredients. The concentrated extract was diluted to 1000 ml using a polysaccharide as a carrier and stored in the refrigerator (Ugwu *et al*., 2013). Determinations of chemical constituents of *Moringa oleifera* extract are shown in table 3.

**Table 3:** Determination of chemical constituents leaf powder of *Moringa oleifera* extract.

| Components (%) | Leaf Powder | Minerals (mg) | Leaf Powder |
|---|---|---|---|
| Moisture | 7.50 | Vitamin B -choline | — |
| Moisture | 8.50 | Vitamin B1 -thiamin | 2.64 |
| Calories Protein | 27.10 | Vitamin B2 -riboflavin | 20.50 |
| Fat | 2.30 | Vitamin B3 -nicotinic acid | 8.20 |
| Carbohydrate | 38.20 | Vitamin C -ascorbic acid | 17.30 |
| Fiber | 19.20 | Vitamin E - tocopherol acetate | 113.00 |
| Minerals (mg) | — | Arginine | 1325.00 |
| Ca | 2.00 | Histidine | 613.00 |
| Mg | 368.00 | Lysine | 1325.00 |
| P | 204.00 | Tryptophan | 425.00 |
| K | 1.32 | Phenylanaline | 1388.00 |
| Cu | 0.57 | Methionine | 350.00 |
| Fe | 28.20 | Threonine | 1188.00 |
| S | 870.00 | Leucine | 1950.00 |
| Oxalic acid | 1600.00 | Isoleucine | 825.00 |
| Vitamin A - B carotene | 16.30 | Valine | 1063.00 |

**Table 4:** Determination of minerals and total carbohydrates in *Aloe vera* extract

| Components | (mg/100ml) | Components | (mg/100ml) |
|---|---|---|---|
| N | 80.65 | Mg | 14.44 |
| P | 06.95 | Zn | 0.028 |
| K | 60.14 | Cu | 0.004 |
| Fe | 0.229 | Mn | 0.027 |
| Ca | 40.00 | Na | 51.12 |
| Total carbohydrates | 10.10 (mg/100mg) | | |

**Table 5:** Determination of Phytohormones in *Aloe vera* extract

| Phytohormones | Concentration(mg/100mg) |
|---|---|
| $GA_3$ | 1.60 |
| IAA | 0.06 |
| ABA | 0.31 |

### Preparation of *Aloe vera* leaves extract (ALE)

*Aloe vera* leaf surface was scraped and the leaf tissue was kept in a flask with added water at equal rate (1:1 by volume), then filtrated. The obtained extract was used for foliar spray at 100 and 150 ml/l (Hanafy *et al*., 2012). (Tables 4 and 5) showed the contents of minerals and Phyto hormones in *Aloe vera* leaf extract.

### Preparation of anatomical specimens

In the second season samples of *F. benjamina* leaves were taken from 5$^{th}$ internode including the midrib at the age of 8 months. Leaf samples were killed and fixed at least 48 hrs. in FAA (10ml formalin, 5ml glacial acetic acid and 85ml ethyl alcohol 70%). The selected materials were washed in 50% ethyl alcohol, dehydrated in a normal butyl alcohol series, embedded in paraffin wax of melting point 56$^{o}$C. Sections were cut in to 15 to 20 µ thick. Safranin- fast green combination method (Sass, 1961) was used for staining. Stained sections were cleared in xylene and mounted in Canada balsam (Willey, 1971). Sections were microscopically examined and photomicrographs were taken. Counts and measurements (µm) of the different leaves were taken and calculated using a micrometer eye piece. The surfaces of *F. benjamina* L. leaves were pictured by using Light Microscope using camera model Leica ICC50 HD at Faculty of Agriculture, Cairo University. The magnification power was expressed by 10×10 for each photograph.

### Statistical analysis

The layout of the experiment is a randomized complete block design with two factors, each treatment contained 18 plants in 3 replicate with six plants. The analysis of variance of data (ANOVA) was carried out according

**Table 6:** Effect of treated with foliar applications with $GA_3$ and natural extracts on histological structures (µm) of *Ficus benjamina* leaves.

| Characters (µm) | Treatments | | | |
|---|---|---|---|---|
| | Control (tap water) | $GA_3$(200ppm) + Yeast extract) (8g/l | $GA_3$(200ppm) + *Moringa oleifera* extract (5ml/l) | $GA_3$(200ppm) + *Aloe vera* extract (100ml/l) |
| Midvein thick. | 410.26 | 599.21 | 457.55 | 414.96 |
| Lamina thick. | 279.46 | 362.37 | 299.26 | 286.72 |
| Palisade tissue thick. | 40.24 | 57.00 | 54.98 | 42.17 |
| Main vascular bundle dimensions | | | | |
| (1) Length | 219.57 | 300.80 | 234.92 | 224.42 |
| (2) Width | 256.80 | 383.47 | 281.55 | 266.64 |
| Mean diameter of vessel | 18.29 | 21.39 | 20.97 | 20.11 |

to Snedecor and Cochran (1976) using MSTAT-C (1989) program based on the least significant difference (L.S.D.) test at $P \leq 0.05$.

**Recorded data**

The following data were recorded after 8 months:

1. **Vegetative growth characters:** plant height (cm), number of leaves/plant, number of branches/plant, root length (cm) and fresh weight of leaves, stems, roots (g/plant).
2. **Anatomical structure:** thickness of; midvein, lamina, palisade tissue and spongy tissue, in addition to main vascular bundles of midvein dimension and the mean vessel diameter.

## Results and discussion

### Vegetative growth characters

Treating *F. benjamina* plants separately either with $GA_3$ at 200ppm or yeast extract at 8g/l gave the highest significant values of plant height, number of leaves/plant, root length and the fresh weights of leaves, stems and roots, in both seasons (Fig. 1 and 3). The only exception was in number of branches/plant where it was increased when the plant were treated with $GA_3$ at 150 ppm and the same dose of yeast extract (8g/l) (Fig. 2).

The lowest values of the above mentioned characters were noticed either in the plants treated with $GA_3$ at 50 ppm or those treated with the *Aloe vera* extract at 100 ml/l, in both seasons. The only exception was the number of leaves /plant where the lowest value was recorded for plants treated with $GA_3$ at 50 ppm and the *Aloe* extract 150 ml/l.

Concerning the combination between $GA_3$ and the other natural extracts at different concentrations, treating plants with $GA_3$ at 200 ppm + yeast extract at 8g/l gave the highest significant values of the all the studied characters, in both seasons. While the lowest values of plant height, number of leaves/plant and the fresh weight of leaves, stems and roots were recorded with $GA_3$ at 50 ppm combined with *Aloe* extract at 100 ml/l. The lowest values of number of branches/plant and the root length were obtained with the combination between $GA_3$ at 150 ppm and *Aloe* extract at 100 ml/l and $GA_3$ at 150 ppm and *Aloe* extract at 150 ml/l, respectively in both seasons (Fig. 2 and 3).

The plant height results, in the present study, were in harmony with those of Abdel-Latif (2006) on *Salvia officinalis,* Abdel-Wahed *et al.,* (2006) on *Euonymus japonicas* plantswhich treated with yeast extract. Hence, gibberellic acid is used to regulate plant growth through increasing cell elongation and cell division of plants. Active dry yeast is a natural safety biofertilizer causes various promotive effects attributed to its character of richness in protein, B-vitamin and natural plant growth regulators such as cytokinins. It also releases $CO_2$ which reflected on improving net photosynthesis. So, using yeast extract play an important role for enhanced plant characters.

Concerning number of leaves per plant, the enhancement effect of yeast extract might be attributed to its influence on metabolism, biological activity, photosynthetic pigments and enzyme activities which in turn encourage vegetative growth especially number of leaves/plant (Wanas, 2002 on *Vicia faba* and El-Sherbeny *et al*., 2007 on *Ruta graveolens*). It is acting as a source of plant growth hormones, carbohydrates, amino acids and vitamins.

Using yeast extract and $GA_3$ increased the plant composition of elements such as N, P, K, Ca, Fe and Zn, these elements are important components (proteins, fats, carbohydrate and chlorophylls) in plant tissues, consequently better growth leads to increase the number of leaves. The former results were in agreement with those obtained EidandAbou-Leila (2006) on croton plants and Hanafy *et al*. (2012) on *Schefflera arboricola*.

The increment occurred in number of branches/plant may be due the application of $GA_3$ not only increased the internode length of the plants but also increased the

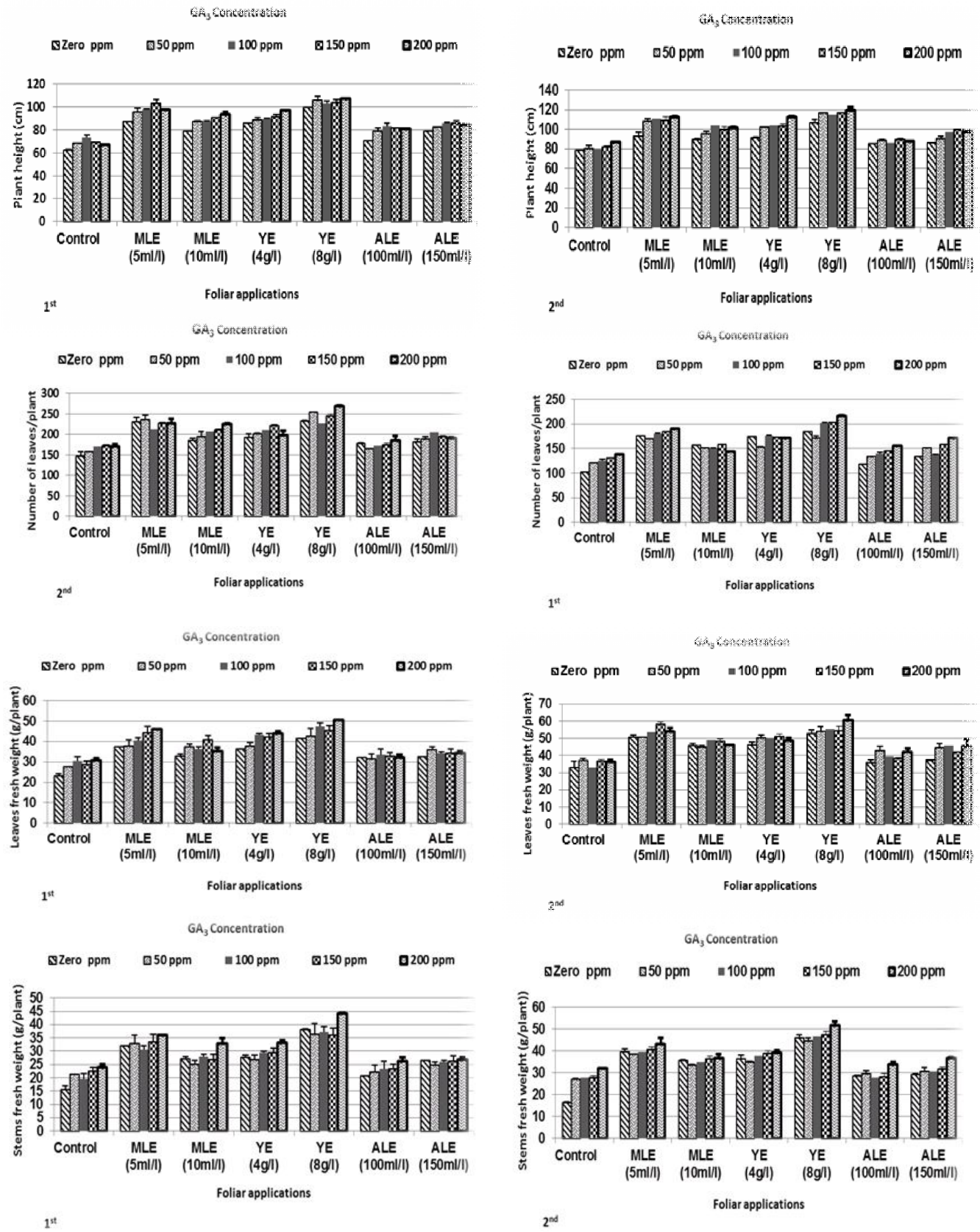


**Fig. 1** Effect of foliar applications with natural extracts and different levels of gibberellic acid on plant height (cm), number of leaves/plant, leaves fresh weight (g/plant) and stems fresh weight (g/plant) of *Ficus benjamina* plants during the 2014/2015 and 2015/2016 seasons. MLE (*Moringa* leaves extracts), YE (Yeast extracts), ALE (Aloe extracts)

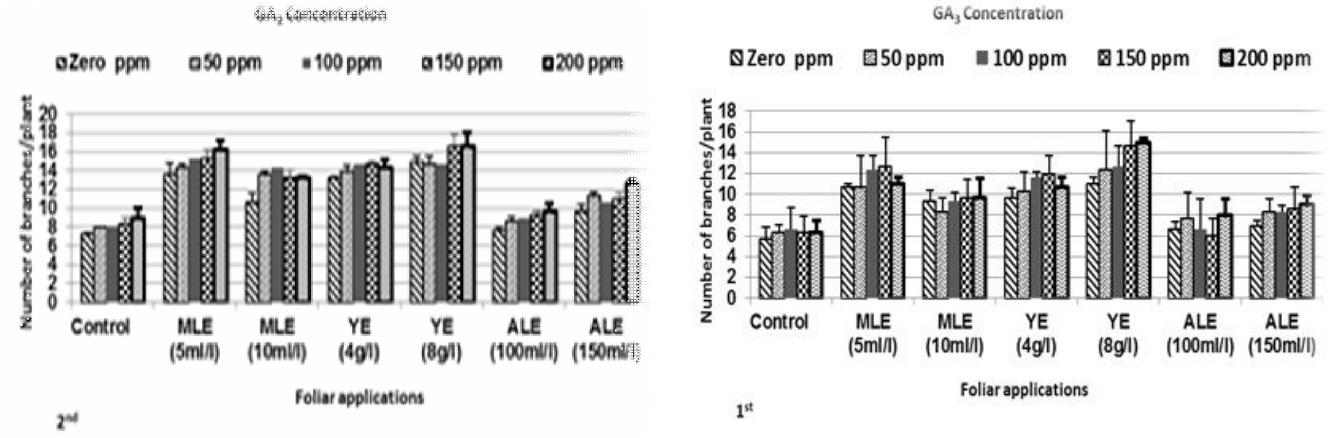


**Fig. 2:** Effect of foliar applications with natural extracts and different levels of gibberellic acid on number of branches/ plant of *Ficus benjamina* plants during the 2014/2015 and 2015/2016 seasons.

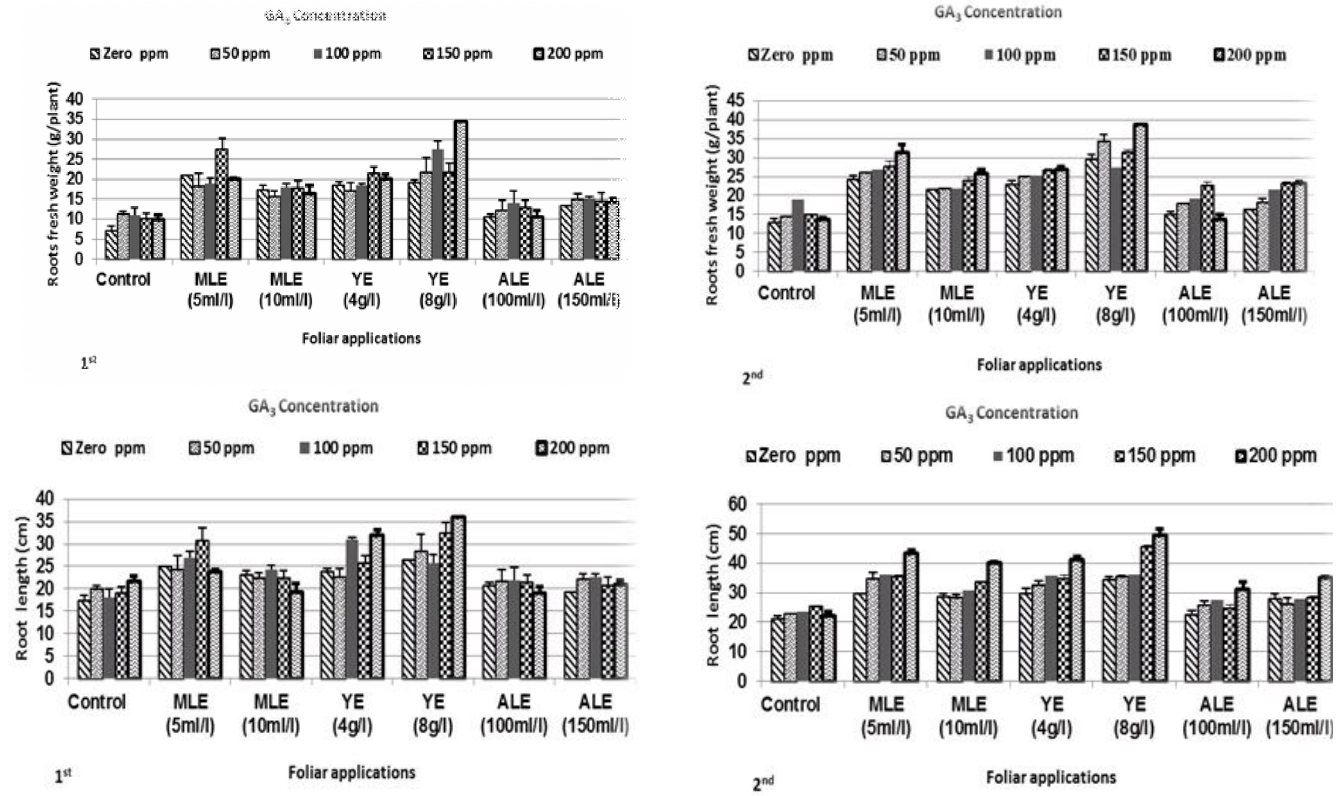


**Fig. 3.:** Effect of foliar applications with natural extracts and different levels of gibberellic acid on roots fresh weight (g/ plant) and root length (cm) of *Ficus benjamina* plants during the 2014/2015 and 2015/2016 seasons.

number of cells, as cell elongation is primarily responsible for the increase in internode length. The present results are in harmony with those of Eid and Mazher (2004) on *Casuarina glauca*, Hussien (2009) on *Cryptostegia grandiflora* and Ibrahim *et al.* (2010), on *Codiaeum variegatum.* The increment in the number of branches formed on *Ficus benjamina* plants treated with yeast extract and gibberellic acid may be due totheenhancing role of natural hormones, including increment in the activity of cytokinins (Mohamed *et al.*, 2005) on *Lilium longiflorum* and (Emam, 2010) on *Polianthes tuberosa*. Stimulating vegetative growth by foliar application of dry yeast may be due to its influence on the availability of the essential nutrients, producing plant growth regulators and suppressing pathogen. The beneficial effect of dry yeast was supported with findings of other workers, *i.e.* Eid (2001) on coriander plant, Naguib and Khalil (2002) on black cumin.

The promotive effect of yeast foliar spray on root length may be due to the accumulation of nutrients and organic matter around plants produced by yeast extract which reflect on plant composition of elements such as

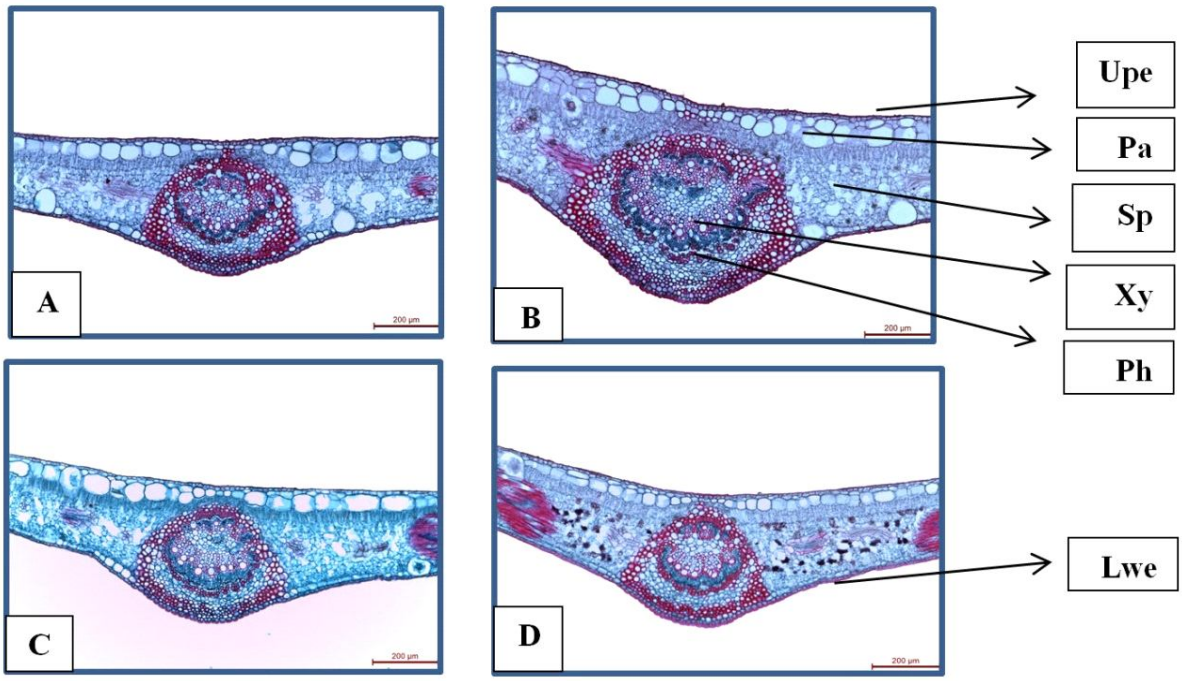


**Fig. 4:** Transverse sections of the midvein portion of the leaf blade of *Ficus benjamina* L. plant sprayed with $GA_3$, yeast, *Moringa* and *Aloe* in the second season.x100

Details: (A) Control, (B) yeast extracts (8g/l) and $GA_3$ (200ppm), (C) *Moringa* extracts (5ml/l) and $GA_3$ (200ppm), (D) *Aloe* extracts (100ml/l) and $GA_3$ (200ppm), Upe: Upper epidermis, Pa: Palisade tissue, Sp: Spongy tissue, Xy: Xylem, Ph: Phloem, Lwe: Lower epidermis.

N, P, K, Ca, Fe and Zn. These elements enhance the cell division and cell elongation which lead to increments in root length. These results agreed with the findings of Ahmed (2002) on *Leucaena leucocephala*, Wahba (2002) on *Oenothera biennis*, Heikal (2005) on *Thymus vulgaris* and Mohamed *et al*., (2005) on *Lilium longiflorum* plants. The maximum root thickness of the plants treated with 200 ppm of gibberellic acid may be due to the greater stem height and branches number occurred which allow the plant to manufactured more nutrients translocate toward the root causing increase in diameter (Gul *et al.,* 2006 on *Araucaria heterophylla*).

The majority increases of the fresh weight of leaves, stems and roots caused by natural extracts and $GA_3$ treatments may be due to the effect of gibberellic acid in activation of photosynthesis process and increase of absorption to water and nutrition, and then lead to the increase of plant growth which increase leaves fresh weight. Similar results were reported by Gul *et al*., (2006) on *Araucaria heterophylla* and Hussein (2009) on *Crypostegia grandiflora*. Figs. 1and 3.

**Anatomical structure of leaves**

Microscopical measurements of certain anatomical characters in transverse sections of *F. benjamina* leaves which sprayed with the combination foliar spray between $GA_3$ at 200 ppm and the natural extracts; active yeast at 8 g/l, *Moringa* at 5 ml and *Aloe* at 100 ml are given in Table 6. The microphotographs illustrating these treatments are shown in Fig. 4.

Data indicated that all the values of the leaf structures of the plant treated with $GA_3$ (200ppm) combined with yeast extract (8g/l) were exceeded those of the control plant by 26.10, 29.70 and 41.70% for the thickness of midvein, lamina and palisade tissue, respectively. Moreover, it increases by 37, 49.30, 17.00% for the length and width of the main vascular bundle and vessel diameter, respectively. While the plant sprayed the combination between $GA_3$ (200 ppm) and *Aloe* extract at 100 ml exhibited the lowest percentages although still greater than the control plants. These percentages were; 1.20, 2.60, 4.80, 2.20, 3.80 and 10.00% for the above mentioned characters. An intermediate values between the previous treatments was noticed when combined $GA_3$ (200 ppm) with *Moringa* at 5 ml.

As far as the authors are aware, information concerning the effect of foliar spray with the combinations of natural extracts on the anatomical structure of the leaf structure of *F. benjamina* plants are very few in the literature.

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