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Promotion of Rooting and Growth of Some Types of Bougainvilleas Cutting by Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhizal Fungi (AMF) in Combination with Indole-3-Butyric Acid (IBA)

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Abstract: ***Divers studies have demonstrated that plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) can stimulate plant growth and more recently that they can increase rooting ability in vegetative material, especially when they are added with auxin. Considering this potential, the objective of this study was to verify the effect of PGPR and AMF in combination with IBA on rooting and growth of some types of bougainvilleas cutting. Three cutting types (tip, middle and basal) were prepared from four bougainvilleas, namely B. glabra var. sanderiana, B. glabra var. variegata, B. spectabilis "Snow White" and B. spectabilis "Yellow Hybrid" in both 2012 and 2013 years. The cuttings were taken from Bougainvillea mother plants in March and treated with three PGPR (Azospirillum brasilense, Pseudomonas fluorescens and Bacillus subtilis) and AMF (Glomus intraradices) in combination with 100 ppm IBA. The all combined treatments of IBA plus PGPR or AMF showed higher rooting percentages than hormone treatment (IBA alone). Among bougainvilleas used, average the highest rooting were observed in B. spectabilis "Snow White" (62.0%), followed by B. glabra var. sanderiana (61.2%), B. spectabilis "Yellow Hybrid" (60.5%) and B. glabra var. variegata (54.7%), respectively. The highest rooting percentages were obtained from basal cuttings treated with 100 ppm IBA plus either G. intraradices, A. brasilense or B. subtilis in all bougainvilleas. Overall, the lowest was observed in the IBA treatment alone. C/N ratio and endogenous root-promoting substances in cutting base were parallel with the rooting ability. The present investigation clearly showed that the combination of PGPR or AMF inoculums and rooting hormone can increase root initiation and potentially increase the quality of rooted cutting produced. Furthermore, the success of root promotion depends on the used strain and genotypic response of Bougainvillea species.***

Keywords: *Bougainvillea*, PGPR, AMF, IBA, Rooting and Type of cutting

1. Introduction

Bougainvillea plant is considered as one of the most usable ornamental climber shrubs. It is very popular nowadays in desert landscaping particularly in seaside tourist villages spread through Egypt. *Bougainvillea* popularity and significance are attributed to its durability, its resistance to adverse conditions and to its wide-range colored bracts which remain for a rather long time of the year. *Bougainvillea glabra* and *B. spectabilis* are widely used species and most *Bougainvillea* cultivars are thought to have originated from them (Bailey, 1914; Singh *et al.*, 2011). Commercial propagation is carried out by semi hardwood stem cuttings and in general, most commercially available species and cultivars are considered difficult to root. Application of plant growth regulators by themselves and in combination with other substances is commonly used to increase adventitious rooting on *Bougainvillea* cuttings (Mahros, 2000; Ahmad *et al.*, 2002; Abdel-Rahman and El-Dosoky, 2010; Memon *et al.*, 2013).

When large quantities of rooted cuttings are required to be produced quickly, propagators look for methods to increase propagation success and decrease the time required for rooting (Armstrong, 2000). The number of roots initiated can influence the length of a production cycle and the quality of rooted cuttings produced. Therefore, alternative techniques that optimize rooting percentage of cuttings will be useful for mass vegetative propagation. The stimulation of adventitious root formation with the use of plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF), currently represents an effective method of vegetative propagation of plants (Diaz-Granados *et al.*, 2009; Abdel-Rahman and El-Dsouky, 2010; Rajan and Radhakrishna, 2013).

Plant growth promoting rhizobacteria (PGPR) have gained world wide importance and acceptance. The mechanisms involved in plant growth promotion by PGPR involve direct and indirect effects. Direct effects occur when PGPR produces substances such as phytohormones. Indirect effects may occur through their ability to prevent or decrease the damage to plants by phytopathogens (Glick *et al.*, 1999). These microorganisms are the potential tools for sustainable agriculture and the trend for future (Siddiqui, 2006). Recent studies confirm that the treatments of cuttings with PGPR such as *Bacillus*, *Azospirillum*, *Pseudomonas* etc. can increase root initiation and increase the quality of rooted cutting produced in some plants because of natural auxin production (IAA) by PGPR (Felker *et al.*, 2005; Li *et al.*, 2005; Ribaudo *et al.*, 2006; Kaymak *et al.*, 2008; Karakurt *et al.*, 2009; Erturk *et al.*, 2010; Abdel-Rahman and El-Dsouky, 2010; Rajan and Radhakrishna, 2013). Although the mechanisms are not

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completely understood, root induction and growth promotion in response to PGPR inoculation may occur by production of phytohormones such as auxins, cytokinins and gibberellins, by inhibition of ethylene synthesis and by mineralization of nutrients by PGPR (Goto, 1990; Steenhoudt and Vanderleyden, 2000; Rajan and Radhakrishna, 2013). In general, growth promotion depends on several mechanisms, and the main effects of PGPR are related to increases in root, stem and branch growth. PGPR can also suppress deleterious or pathogenic microorganisms or stimulate the association of mycorrhizal fungi and *Rhizobium* sp. (Mahaffe and Kloepper, 1994).

Mycorrhizae are symbiotic associations between plant roots and certain soil fungi that can enhance plant productivity (Pfleger and Linderman 1994). Plants with mycorrhizae are potentially more effective at nutrient and water acquisition (Koide, 1991), less susceptible to disease (Linderman, 1994), and can be more production under certain stressful environmental growing conditions than plants without mycorrhizae (Miller and Jastrow, 1992). Vesicular-arbuscular mycorrhizal fungi (VAMF) are one type of mycorrhizal fungi that are commonly associated with the roots of plants. The benefits from root colonization by VAMF are thought to be highest when colonization occurs as early as possible during plant growth (Chang, 1994). In the propagation of plant from cuttings, this means that maximum benefits from VAMF colonization would be obtained if inoculum is present during adventitious root formation. The addition of mycorrhizal fungi into the rooting substrate during cutting propagation can increase rooting in different plants (Scagel, 2001; Thanuja *et al.*, 2002; Scagel *et al.*, 2003; Druege *et al.*, 2006; Diaz-Granados *et al.*, 2009). Arbuscular mycorrhizal fungi (AMF) are known to enhance plant growth through increased nutrient and water uptake and growth hormone production. The production of growth hormones such as auxins, gibberellin like substances and cytokinins has been well demonstrated (Barea and Azcon-Aguilar, 1982). AM fungi are known to increase rooting due to the production of these growth hormones (Scagel and Linderman, 1998) and polyphenolic compounds which decrease auxin oxidation (Mitchell *et al.*, 1986).

In addition, several studies have shown that rooting of cuttings inoculated with PGPR and AMF can be accelerated by exogenous IBA application (Scagel, 2001; Scagel *et al.*, 2003; Eşitken *et al.*, 2003; Karakurt *et al.*, 2009). Abdel-Rahman and El-Dsouky (2010) found that treatment of *Bougainvillea glabra* var. *sanderiana* cuttings with 100 ppm IBA plus *Bacillus subtilis* at 10 ml/pot broth culture containing 10^8 CFU/ml is more effective in increasing rooting ability and more quality rooting compared to IBA or *B. subtilis* alone. Scagel (2001) showed that addition of AMF (*Glomus intraradices*) inoculum into the rooting substrate during cutting propagation would increase the quantity of roots and the quality of rooted cuttings for five different cultivars of miniature rose. He added that the combination of hormone treatment (IBA or NAA) and AMF inoculum in the rooting substrate produced a better percentage of rooted cuttings with more roots than cuttings treated only with hormone.

With regard to these traits, some known bacterial stains (*Azospirillum brasilense*, *Pseudomonas fluorescens* and *Bacillus subtilis*) and arbuscular mycorrhizal fungi (*Glomus intraradices*) in combination with IBA were investigated with the aim to improve rooting and growth of some types of bougainvilleas cutting.

2. Material and Methods

This study was carried out at the Horticulture Farm, Faculty of Veterinary and Agricultural Science, El-Zawia University, Libya, during the two successive seasons of 2012 and 2013 to examine the effectiveness of plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) in combination with indole-3-butyric acid (IBA) on rooting, root and shoot growth of some types of bougainvilleas cutting.

Active strains of *Azospirillum brasilense*, *Pseudomonas fluorescens*, *Bacillus subtilis* and arbuscular mycorrhizal fungi (*Glomus intraradices*) were obtained from the Unit of Biofertilizers, Fac. Agric., Ain Shams Univ., Shobra El-Kheima, Egypt. The rooting substrate was inoculated with bacterial suspensions (10^8 CFU/ml) or AMF before sticking *Bougainvillea* cuttings. *Azospirillum brasilense*, *Pseudomonas fluorescens* and *Bacillus subtilis* inoculations were applied at a rate of 10 ml/pot. The inoculation with AMF was placed in the rooting substrate at 25 spores/pot.

Four healthy bougainvilleas plants namely *B. glabra* var. *sanderiana*, Hort., *B. glabra* var. *variegata*, Hort., *B. spectabilis* "Snow White", Willd., *B. spectabilis* "Yellow Hybrid", Willd. were used as source of cuttings. On March 15^{th}, three cutting types (tip, middle and basal) were prepared from one year old branches of bougainvilleas, each of 15 cm long; with diameter ranges of 0.5-0.6, 0.8-0.9 and 1.1-1.2 cm for tip, middle and basal cuttings respectively, during the tow seasons of this investigation.

Indole-3-butyric acid (IBA) solution at concentration of 100 ppm was used for soaking 2-3 cm of cutting base for 20h for all bougainvilleas. The combined treatments were applied by sticking IBA-treated cuttings into the rooting substrate which contains inoculums of *A. brasilense*, *P. fluorescens*, *B. subtilis* and *G. intraradices*. Cuttings in the control group were treated with 100 ppm IBA alone.

The experiment was arranged in a split-split-plot design, with three replicates. *Bougainvillea* species (*B. glabra* var. *sanderiana*, *B. glabra* var. *variegata*, *B. spectabilis* "Snow White", *B. spectabilis* "Yellow Hybrid") represented in the main plots, meanwhile type of cuttings (tip, middle and basal) and beneficial microorganisms (IBA, IBA + *A. brasilense*, IBA + *P. fluorescens*, IBA + *B. subtilis* and IBA + *G. intraradices*) represented in the sub-plots and sub-sub-plots, respectively. Each experimental unit contained 10 cuttings were planted in a plastic pot of 20 cm diameter filled with perlite and peat moss (1:1 in volume). The experiment was done under plastic house and covered by tightly polyethylene film to maintain high relative humidity.

After three months from the commencement of the rooting trials, cuttings were dug up, cleaned and data were recorded

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on: rooted cuttings percentage, root number, root length, branch number and branch length per cutting. One centimeter sample of the basal end representing each type of cutting were taken and dried for determination of carbohydrates and nitrogen. Total carbohydrate content was colorimeterically determined with the anthrone sulphuric acid method; Fales (1951). Total nitrogen content was determined by semi-micro Kjeldahl method; Black *et al.* (1982). Then, carbon/nitrogen ratio (C/N ratio) was calculated.

Bioassay test to determine endogenous rooting co-factors using the mung bean cuttings was used as indicator of promoters and inhibitors in cuttings; ethanol extracts of each cutting types for the method described by Fadl and Hartmann (1967). Data obtained during the tow seasons of the study were statistically analyzed according to Steel and Torrie (1982) using the MSTAT computer software.

3. Results and Discussion

3.1 Rooting percentage

Data presented in Table (1) indicate that the combination of rooting hormone (IBA) and PGPR or AMF inoculums in the rooting substrate increased percentage of rooted cuttings for both species of *Bougainvillea* compared to cuttings that only received IBA treatment. Among the PGPR and AMF used, the best rooting percentages were observed in cuttings treated with *Azospirillum brasilense*, *Bacillus subtilis* and *Glomus intraradices* in combination with IBA. However, no significant differences in rooting percentage were achieved among *A. brasilense*, *B. subtilis* and *G. intraradices*. The increases were 67.4, 57.5, 64.9 and 67.9% in rooting percentage for IBA + *A. brasilense*, IBA + *P. fluorescens*, IBA + *B. subtilis* and IBA + *G. intraradices*, respectively over the general control (IBA alone) as average mean of both seasons as illustrated in Fig. (1). These results are in agreement with several investigations; Scagel (2001) on miniature rose, Scagel *et al.* (2003) on *Taxus* x *media* "Hicksii", Erturk *et al.* (2008) on *Camellia sinensis*, Erturk *et al.* (2010) on *Actinidia deliciosa* and Abdel-Rahman and El-Dsouky (2010) on *Bougainvillea glabra* var. *sanderiana*. They found that combined IBA-bacteria or IBA-mycorrhizae treatments enhanced rooting of cuttings more than treatment with IBA alone. Several investigators demonstrated that *A. brasilense*, *P. fluorescens* and *B. subtilis* had the capacity of IAA production (Ribaudo *et al.*, 2006; Teixeira *et al.*, 2007; Erturk *et al.*, 2008; Erturk *et al.*, 2010; Rajan and Radhakrishna, 2013). It is suggested that the stimulation of rooting by PGPR may be due to the production of IAA by the PGPR (Erturk *et al.*, 2008 and 2010; Rajan and Radhakrishna, 2013). AM fungi are known to increase rooting due to the production of growth hormones such as auxins, gibberellin like substances and cytokinins (Scagel and Linderman, 1998) and polyphenolic compounds which decrease auxin oxidation (Mitchell *et al.*, 1986). Our results indicate that the rooting of bougainvilleas cutting treated with IBA can be accelerated by adding PGPR and AMF inoculums into the rooting substrate.

The present results also indicate that the rooting of cuttings varied with the *Bougainvillea* species. Generally, *B. glabra* var. *sanderiana*, *B. spectabilis* "Snow White" and *B. spectabilis* "Yellow Hybrid" cuttings rooted better than *B. glabra* var. *variegata* cuttings when they were treated with IBA alone or in combination with PGPR and AMF (Table 1 and Fig. 2). These results agreed with those reported by Mahros (2000) and Memon *et al.* (2013) on *Bougainvillea*. They reported that the success of root promotion depends on the genotypic response of plant species. The cultivar-specific responses could be a result of specific interactions between the beneficial microbes and traits specific to each cultivar such as environmental, nutritional, or hormonal requirements for optimal rooting (Scagel, 2001).

Concerning the effect of cutting types on rooting of the four bougainvilleas (Table 1 and Fig. 2), it is obvious that basal cutting showed a higher rooting percentage (80.7%) compared to the tip and middle ones (38.6 and 64.9%; respectively). These results are in accordance with those obtained by Mahros (2000), Ahmad *et al.* (2002) and Abdel-Rahman and El-Dsouky (2010) on *Bougainvillea*. The higher rooting of basal cuttings may be determined by higher resistance of these cuttings to higher temperature during the propagation. In addition, basal cuttings proved to be more resistance to botrytide, which often attacked the less mature apical cuttings under the plastic cover (Henselosà *et al.*, 2002).

The interaction effects among the different treatments (Fig. 2) showed that the highest rooting percentages were obtained from basal cuttings treated with 100 ppm IBA plus either *A. brasilense* (87.5%), *B. subtilis* (86.1%) or *G. intraradices* (87.2 %).

3.2 Root and Shoot Characteristics

It is evident from the data in Tables (1 & 2) that application of rooting hormone (IBA) and adding PGPR or AMF inoculums into the rooting substrate of bougainvilleas cutting significantly increased root number, root length, branch number and branch length per cutting compared to cuttings that only treated with rooting hormone (IBA). Generally, treatment of bougainvilleas cutting with IBA plus either *A. brasilense*, *B. subtilis* or *G. intraradices* was more effective on increasing root and shoot growth characteristics than IBA + *P. fluorescence* and IBA treatments. The increments were 100.9, 82.6, 100.9 and 104.6% in root number, 68.6, 57.1, 74.3 and 82.9% in root length, 23.1, 15.4, 30.8 and 23.1% in branch number, and 49.6, 41.8, 51.3 and 53.4% in branch length for IBA + *A. brasilense*, IBA + *P. fluorescens* IBA + *B. subtilis* and IBA + *G. intraradices*, respectively over the IBA treatment alone (Fig. 1). Similar results were obtained by Scagel (2001), Scagel *et al.* (2003), Erturk *et al.* (2008), Karakurt *et al.* (2009) and Abdel-Rahman and El-Dosoky (2010), who found that the combination of hormone treatment and PGPR or AMF inoculums in the rooting substrate increased the number of roots and the quality of rooted cuttings when compared to cuttings that only received hormone treatment. The increment in root and shoot growth measurements as a result of PGPR and AMF inoculations can be attributed to the growth hormones production and increased nutrient and water uptake by PGPR and AMF (Barea and Azcon-Aguilar, 1982; Steenhoudt and Vanderleyden, 2000; Erturk *et al.*,

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2010; Rajan and Radhakrishna, 2013). Thanuja *et al*. (2002) reported that mycorrhizal inoculation increased the P content. P, a constituent of phosphonucleotides which tend to increase cell division (Black, 1965), might have increased the root growth. One of the more characteristic effects of PGPR and AMF is an increased elongation rate, and perhaps initiation rate of lateral roots resulting in more branched root system architecture (Lifshitz *et al*., 1987).

Concerning, the difference between bougainvilleas species, it was found *Bougainvillea glabra* var. *sanderiana*, *B. spectabilis* "Snow White" and *B. spectabilis* "Yellow Hybrid" cuttings produced the best root number, root length, branch number and branch length per cutting. Meanwhile, *B. glabra* var. *variegata* cuttings produced the lowest limit in this concern (Tables 1 & 2). These results are in harmony with those obtained by Mahros (2000) and Memon *et al*. (2013) on *Bougainvillea*.

Generally, it can clearly be noticed that propagation of bougainvilleas by basal cuttings resulted in the best number and length of roots, which reflected in improving the quality and growth of shoots compared to the middle and tip ones. The present results are in accordance with those obtained by several investigators; Ahmad *et al*. (2002) and Abdel-Rahman and El-Dsouky (2010), who found that propagation of *Bougainvillea* by basal cuttings resulted in the best root and shoot growth measurements of rooted cuttings compared with middle and tip ones.

Apparently, the highest root and shoot growth measurements were obtained by the basal cuttings of bougainvilleas, which had been treated with IBA plus either *A. brasilense*, *B. subtilis* or *G. intraradices* (Fig. 3 & 4). These results are in harmony with those obtained by Scagel (2001), Scagel *et al*. (2003) Erturk *et al*. (2008), Karakurt *et al*. (2009) and Abdel-Rahman and El-Dsouky (2010), who found that cuttings treated with the combination of rooting hormone and PGPR or AMF inoculums had greater root and shoot growth when compared to cuttings that only received hormone treatment. Increases in root and shoot growth in response to adding PGPR and AMF inoculums into the rooting substrate can potentially decrease the amount of time for cuttings to attain an adequate amount of roots for transplanting.

Accordingly, the best root and shoot growth of the previous treatments could be attributed to large size of root system which absorbs high rates of nutrients and water reflected on more vegetative growth. Hartmann *et al*. (2002) explained that large root size on cutting enhance shoot growth rate. Mertens and Wright (1978) explained that the rhythmic growth of woody plants was occurred by absorbing nitrogen in roots which reacts with carbohydrates to promote their development. Subsequently more nutrient absorption which transported to the shoot where it combines with carbohydrates to form protein and promote shoot growth.

3.3 C/N Ratio

Results presented in Table (2) indicate that bougainvilleas cutting treated with the combination of IBA and PGPR or AMF inoculums increased C/N ratio in basal part of stem cuttings, where the root initials are formed, than hormone treatment (IBA alone). Maximum C/N ratio in basal portion of the cuttings was obtained as a result of application of IBA in combination with A. *brasilense*, *B. subtilis* or *G. intraradices* inoculums. These results are in accordance with those obtained by Scagel (2001), Thanuja *et al*. (2002) and Abdel-Rahman and El-Dsouky (2010), who found that PGPR and AMF inoculations increased C/N ratio in cutting tissues which lend to increase rooting ability. The increment in C/N ratio in bases of bougainvilleas cutting as a result of application of IBA and adding PGPR or AMF inoculums into the rooting substrate might be due to the production of IAA by PGPR and AMF, which stimulated adventitious root formation and resulted in better absorption of nutrients and water from the soil as well as increasing of vegetative growth. A correlation was found among carbohydrate content, total nitrogen, growth promoters of cutting bases and the rooting response in avocado cuttings (Reuveni and Raviv, 1981). Many changes in metabolism are known to occur during adventitious root formation including changes in amino acids and proteins important for enzyme function and nitrogen metabolisms, and changes in carbohydrates (Hassig, 1986). With miniature roses, Scagel (2001) has tracked differences in total amino acid, protein, and carbohydrates in cuttings, and compared how mixing AMF into the rooting substrate changes composition during the initial stages of rooting. He found that differences in protein and amino acids between cuttings exposed to inoculum and cuttings with no inoculum were detectable within two to four days after cutting while differences in carbohydrates were detectable within four to seven days after cutting.

The recorded data indicated that C/N ratio in basal part of stem cuttings of both *Bougainvillea glabra* var. *sanderiana*, *B. spectabilis* "Snow White" and *B. spectabilis* "Yellow Hybrid" was higher than tissues of *B. glabra* var. *variegata* cuttings. So, it could be pointed out that the highest rooting ability of bougainvilleas cutting is paralleled to high C/N ratio in their bases.

On the other hand, C/N ratio in tissues of bougainvilleas cutting showed that basal cuttings had the highest C/N ratio compared to the tip and middle ones. These results are in agreement with those obtained by Mahros (2000) and Abdel-Rahman and El-Dsouky (2010).

4. Endogenous Rooting Co-Factors

The most noticed interesting relationship between the basal contents of promoters and rooting behavior of bougainvilleas cutting was clearly shown in Fig. (5) and Table (1). Cutting extracts of bougainvilleas treated with IBA and PGPR or AMF inoculums increased number of roots per cow-pea cutting compared to cutting extracts which only treated with IBA. Generally, the highest number of roots per cow-pea cutting was obtained from cutting extracts treated with IBA plus either *A. brasilense*, *B. subtilis* or *G. intraradices*. These increases in number of roots per cow-pea cutting as a result of the combination of IBA and PGPR or AMF inoculums may be due to the role of PGPR and AMF in producing of IAA. Many studies have confirmed that *A. brasilense*, *P. fluorescens*, *B. subtilis* and *G. intraradices* had the capacity of IAA production,

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increased rooting percentage and root biomass (Barea and Azcon-Aguilar, 1982; Li *et al*., 2005; Ribaudo *et al*., 2006; Teixeira *et al*., 2007; Erturk *et al*., 2008; Erturk *et al*., 2010; Rajan and Radhakrishna, 2013). Our results indicate that the effect of IAA depends not only on the quantity produce by PGPR and AMF but also on the endogenous level of the hormone in the plant and exogenous auxin application. Several investigators reported that application of IBA in combination with PGPR or AMF inoculums was more effective on increasing of rooting percentage, root and shoot growth compared to the hormone treatment (Scagel, 2001; Scagel *et al*., 2003; Erturk *et al*., 2008; Karakurt *et al*., 2009; Abdel-Rahman and El-Dsouky, 2010).

In the present investigation (Fig. 6) clearly shows that cutting extracts of both *Bougainvillea glabra* var. *sanderiana*, *B. spectabilis* "Snow White" and *B. spectabilis* "Yellow Hybrid" showed the highest number of roots per cow-pea cutting, compared to *B. glabra* var. *variegata* which has high content of inhibitors in their cutting extracts. So, it could be said that the highest rooting ability of cuttings for both species of *Bougainvillea* depends on the level of growth promoters in the extracts of basal portion of cutting where the new roots are performed.

On the other hand, basal cutting extracts of the four bougainvilleas contained high endogenous promoters that reflected as increase in number of roots per cow-pea cutting comparing with that of tip and middle ones (Fig. 6). Similar results were obtained by Abdel-Rahman and El-Dsouky (2010), who found that basal cuttings of *Bougainvillea* treated with IBA plus *Bacillus subtilis* rooted better and contained high promoters than tip and middle ones. Inoculation of PGPR and AMF perhaps resulted in higher levels of endogenous rooting hormone in basal cuttings and hence better rooting as compared to middle and tip cuttings (Douds *et al*., 1995).

The present results generally showed that the rooting percentages are similar to the rooting promoter's activity. It seems likely that differences between the four bougainvilleas in rooting response may be related to this content of rooting co-factors contained in cuttings. Other investigators have reported a similar relationship between rooting ease and amount of endogenous root-promoting substances; Lee *et al*. (1969). They concluded that some endogenous rooting co-factors, other than auxin, which control rooting, are believed to occur in easy-to-root cuttings of some genera, but to be present in a smaller amount or absent in the difficult-to-root ones. They added that the presence of some root-promoting substances named rooting co-factors, in the extracts obtained from some woody ornamental cuttings were responsible for its high rooting. Rooting co-factors were related to rooting ability.

As conclusion, this study demonstrated that the PGPR (*Azospirillum brasilense*, *Bacillus subtilis* and *Pseudomonas fluorescens*) and AMF (*Glomus intraradices*) have potential to promote root formation in *Bougainvillea* cuttings in the mass clonal propagation. It seems that application of rooting hormone (IBA) and adding PGPR or AMF inoculums into the rooting substrate are more effective on increasing rooting ability, root and shoot growth compared to cuttings which only treated with IBA. Among PGPR and AMF used, as mentioned before, *A. brasilense*, *B. subtilis* and *G. intraradices* are more effective on rooting, root and shoot growth of the four bougainvilleas cutting than *P. fluorescens*. Basal cuttings of *Bougainvillea* responded better to the inoculations of both PGPR and AMF than tip and middle ones. The stimulation of rooting, root and shoot growth by PGPR and AMF is could be result of production of growth hormones by the bacteria and mycorrhizae. Increases in root and shoot growth in response to adding PGPR or AMF inoculums into the rooting substrate in combination with IBA can potentially decrease the length of a production cycle and increased the quality of rooted cuttings produced. These results can also be important for the use of these PGPR and AMF to reduce chemical treatments and the cost of propagation in nurseries, control soil pathogens, and to multiply organic nursery materials.

Table 1: Rooting percentage, number of main roots and the highest root length (cm) per cutting in the four bougainvilleas as affected by type of cuttings and beneficial microorganisms combined with IBA after 3 months from planting during 2012 and 2013 seasons

Treatments	2012 season			2013 season		
	Rooting %	Root number	Root length (cm)	Rooting %	Root number	Root length (cm)
Bougainvillea cultivars:						
B. glabra var. *sanderiana*	62.4 a	20.3 ab	5.4 bc	59.9 ab	19.0 a	5.3 ab
B. glabra var. *variegata*	57.4 b	16.5 b	5.0 c	52.0 c	16.1 b	4.7 b
B. spectabilis "Snow White"	63.2 a	22.2 a	6.1 a	60.7 a	20.4 a	5.9 a
B. spectabilis "Yellow Hybrid"	62.6 a	21.7 a	5.6 b	58.4 b	18.5 a	5.6 a
L.S.D. at 0.05	3.5	4.0	0.5	1.8	2.0	0.7
Type of cuttings**:**						
Tip	38.6 c	13.1 c	5.3 b	35.6 c	12.7 c	5.1 b
Middle	64.9 b	19.5 b	5.5 b	60.1 b	17.6 b	5.3 b 5.7 a
Basal	80.7 a	28.0 a	5.8 a	77.6 a	25.2 a	0.3
L.S.D. at 0.05	2.2	1.6	0.3	1.9	1.3	

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Beneficial microorganisms:						
Control (IBA100 ppm)	40.9 c	11.7 c	3.6 d	37.6 c	10.0 c	3.3 d
IBA + *Azospirillum brasilense*	67.9 a	22.8 a	6.0 b	63.7 a	20.9 a	5.8 b
IBA + *Pseudomonas fluorescens*	63.5 b	20.7 b	5.5 c	60.2 b	19.1 b	5.4 c
IBA + *Bacillus subtilis*	66.5 a	22.9 a	6.1 ab	63.3 a	20.8 a	6.0 ab
IBA + *Glomus intraradices*	68.1 a	22.7 a	6.4 a	63.9 a	21.8 a	6.3 a
L.S.D. at 0.05	2.6	1.4	0.3	2.4	1.1	0.3

Table 2: Number of branches, branch length per plant and means of C/N ratio in cutting base of bougainvilleas as affected by type of cuttings and beneficial microorganisms in combination with IBA after 3 months from planting during 2012 and 2013 seasons.

Treatments	2012 season			2013 season		
	Branch number	Branch length (cm)	C/N ratio	Branch number	Branch length (cm)	C/N ratio
Bougainvillea cultivars:						
B. glabra var. *sanderiana*	1.5 ab	34.9 b	18.9 c	1.6 a	33.7 a	18.4 b
B. glabra var. *variegata*	1.4 b	28.8 c	17.4 d	1.4 b	26.6 b	16.7 c
B. spectabilis “Snow White”	1.5 ab	36.3 a	20.1 a	1.6 a	34.3 a	19.5 a
B. spectabilis “Yellow Hybrid”	1.6 a	36.1 a	19.4 b	1.6 a	33.9 a	18.9 b
L.S.D. at 0.05	0.1	1.1	0.4	0.1	2.2	0.5
Type of cuttings**:**						
Tip	1.3 c	25.2 c	15.8 c	1.3 c	22.7 c	15.5 c
Middle	1.5 b	34.6 b	18.9 b	1.5 b	33.0 b	18.6 b
Basal	1.7 a	42.2 a	22.2 a	1.7 a	40.7 a	21.1 c
L.S.D. at 0.05	0.1	0.9	0.3	0.1	1.5	0.3
Beneficial microorganisms:						
Control (IBA100 ppm)	1.3 b	24.6 c	16.7 d	1.3 c	22.9 c	16.0 d
IBA + *Azospirillum brasilense*	1.6 a	36.4 a	19.7 b	1.6 ab	34.7 a	19.1 b
IBA + *Pseudomonas fluorescens*	1.5 a	34.7 b	18.2 c	1.5 b	32.4 b	17.4 c
IBA + *Bacillus subtilis*	1.6 a	37.1 a	20.8 a	1.7 a	34.9 a	20.1 a
IBA + *Glomus intraradices*	1.6 a	37.3 a	19.4 b	1.6 ab	35.7 a	19.3 b
L.S.D. at 0.05	0.1	1.1	0.4	0.1	1.2	0.5

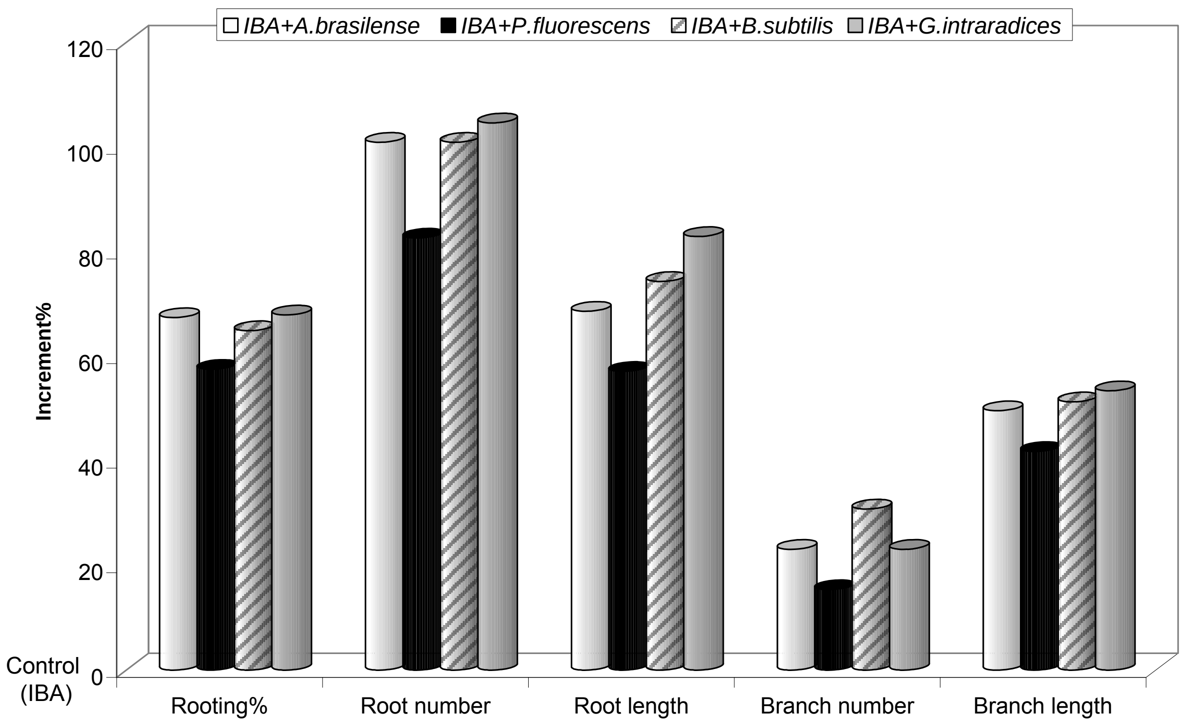

Figure 1: General means of increment percentages of rooting capacity, root number, root length, branch number and branch length of *Bougainvillea* cuttings over the control (IBA) at 3 months planting after as affected by PGPR and AMF inoculums in combination with IBA.

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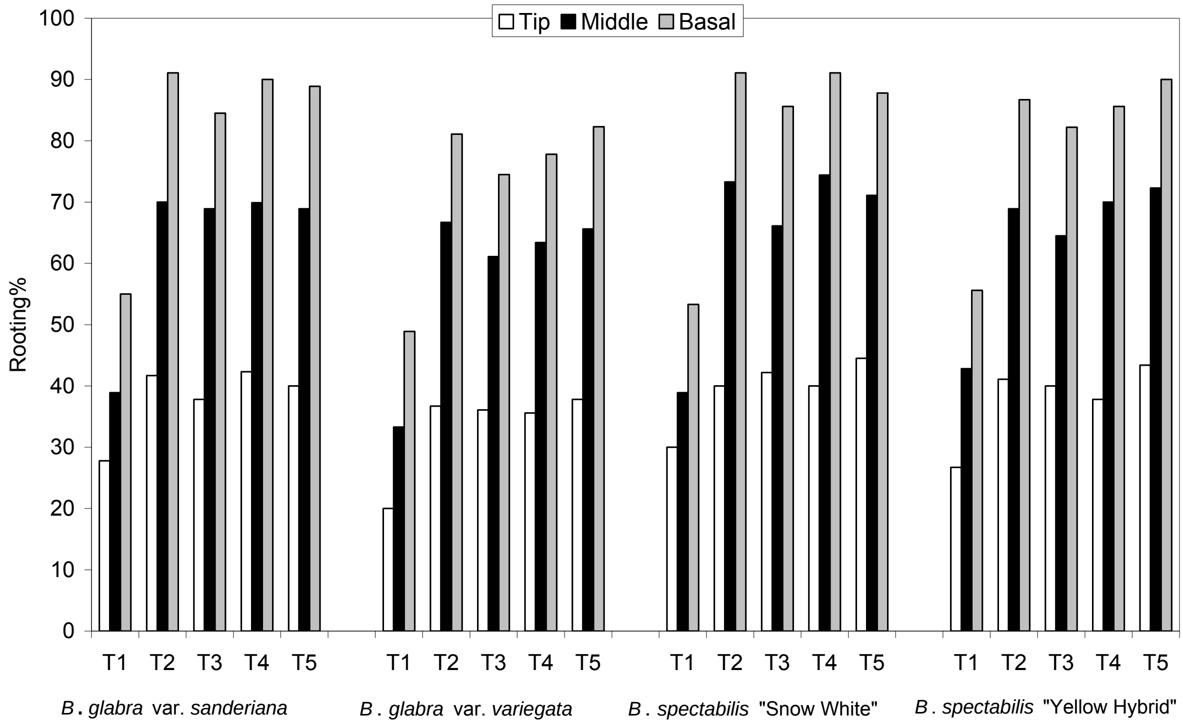
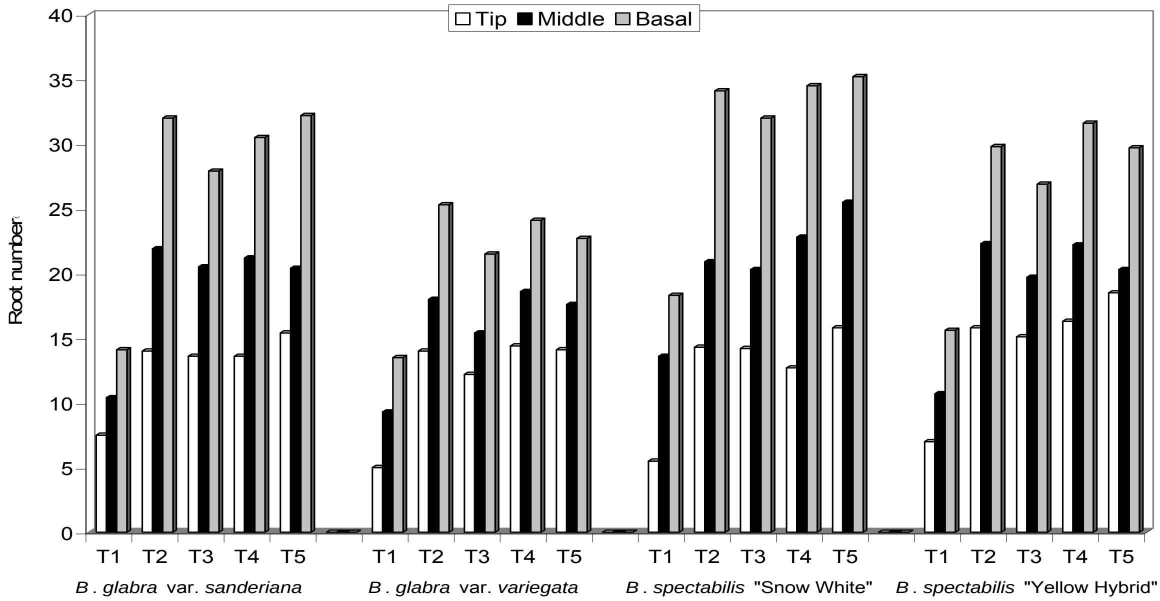


T1= IBA T2= IBA + *A. brasilense* T3= IBA + *P. fluorescens* T4= IBA + *B. subtilis* T5= IBA + *G. intraradices*

Figure 2: Influence of PGPR and AMF inoculums in combination with IBA on rooting percentage of some types of bougainvilleas cutting.

a. Root number



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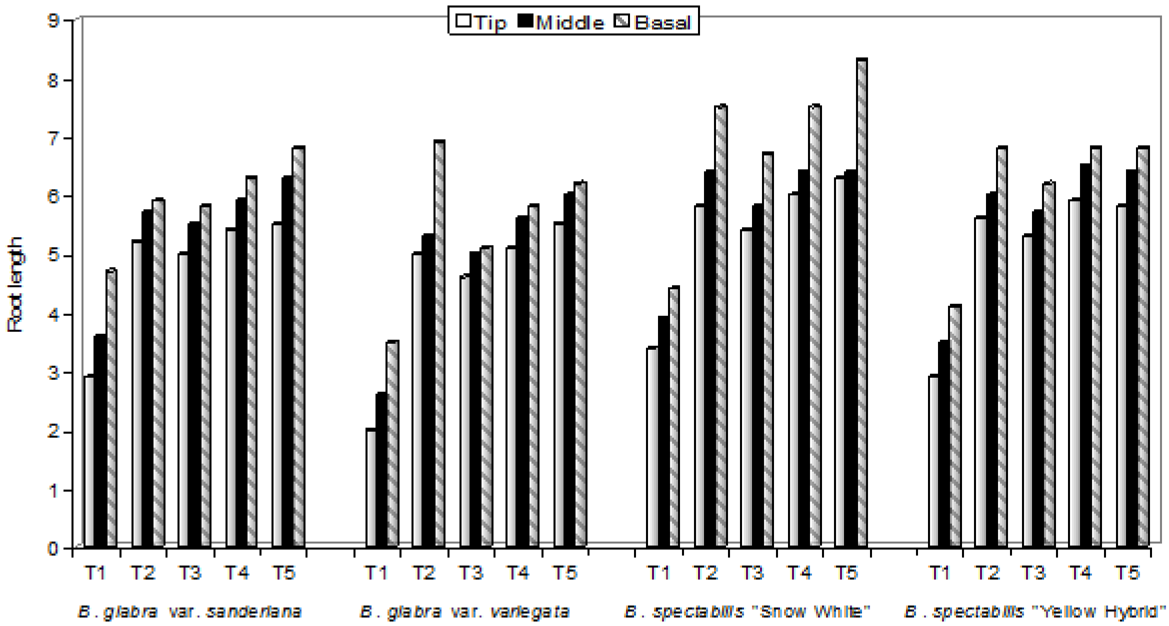
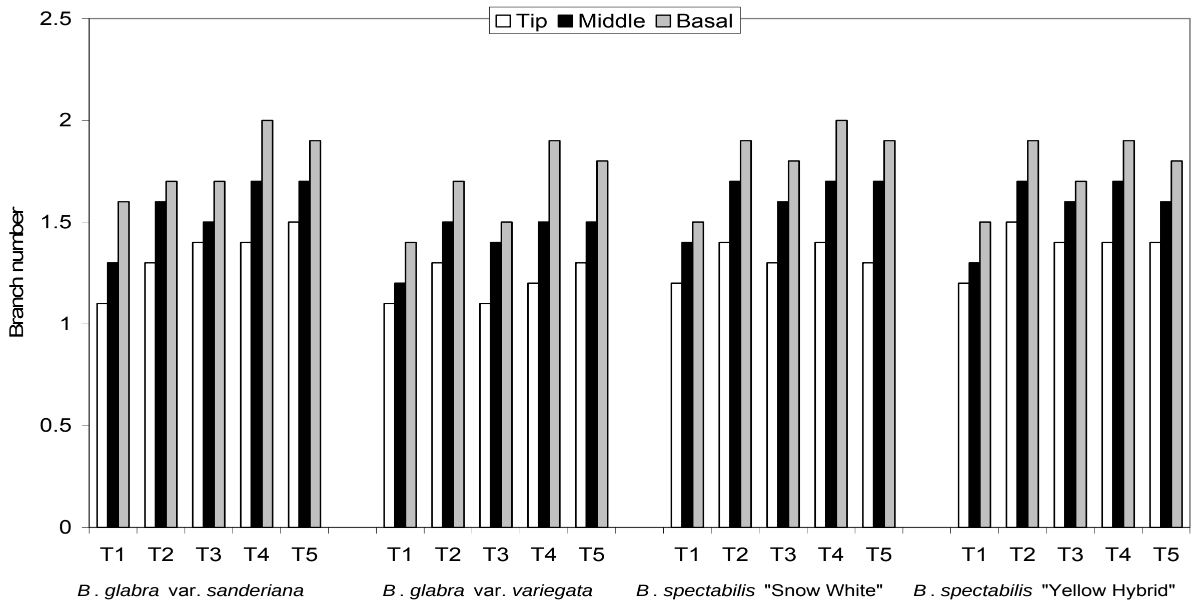
b. Root length



T1= IBA T2= IBA + *A. brasilense* T3= IBA + *P. fluorescens* T4= IBA + *B. subtilis* T5= IBA + *G. intraradices*

Figure 3: Influence of PGPR and AMF inoculums in combination with IBA on root growth of some types of bougainvilleas cutting.

a. Branch number



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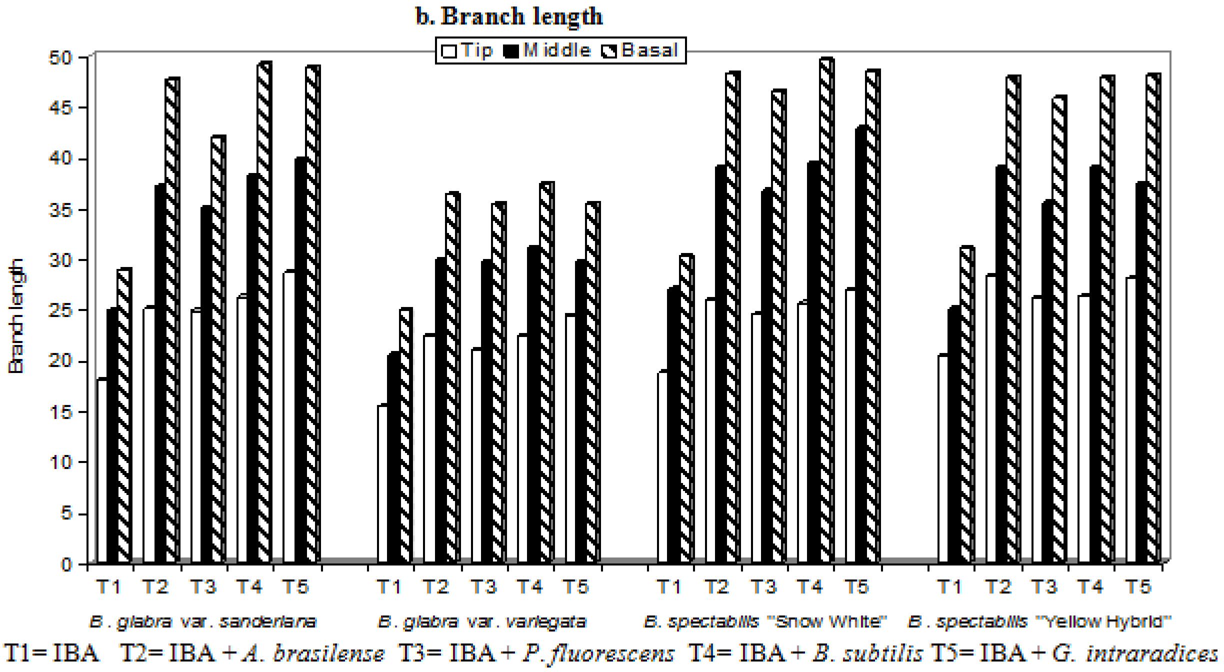
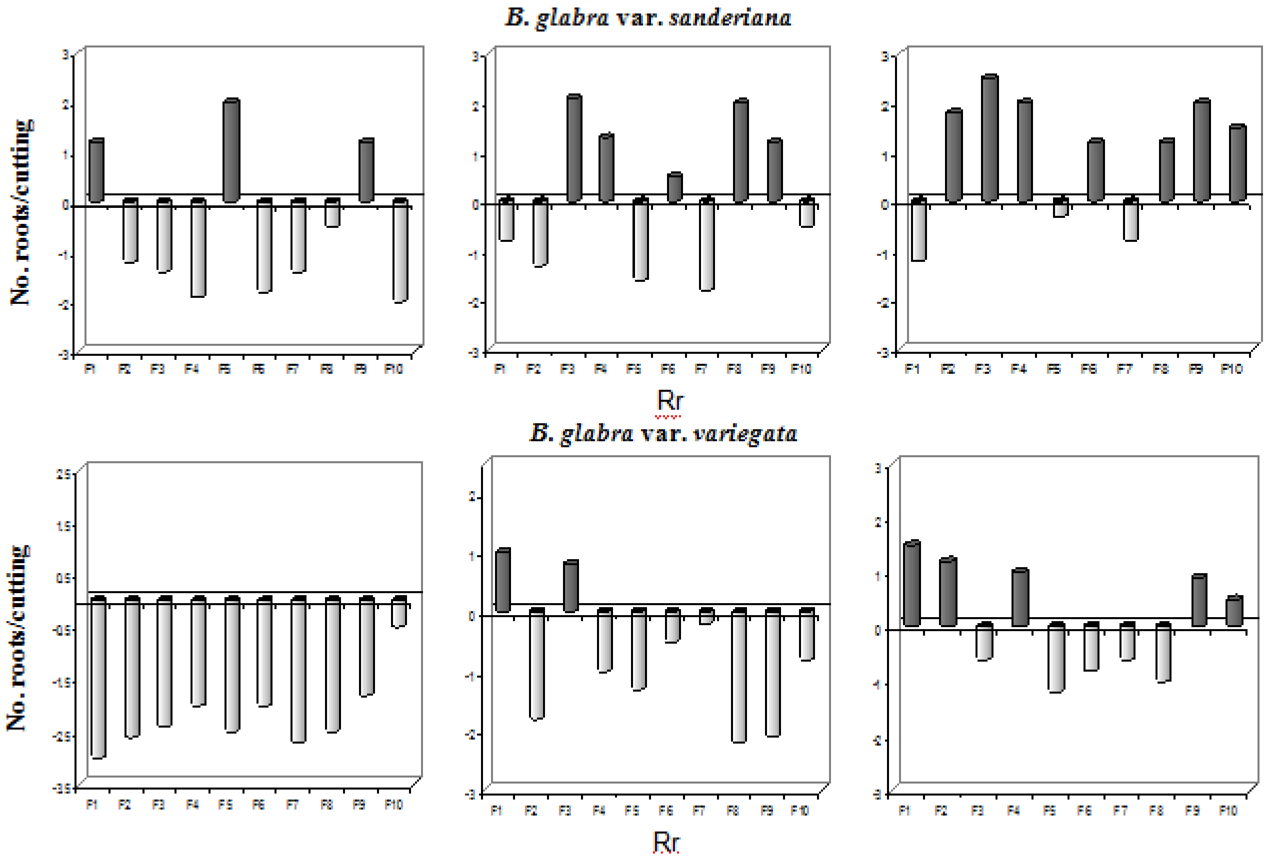


Figure 4: Influence of PGPR and AMF inoculums in combination with IBA on shoot growth of some types of bougainvilleas cutting.



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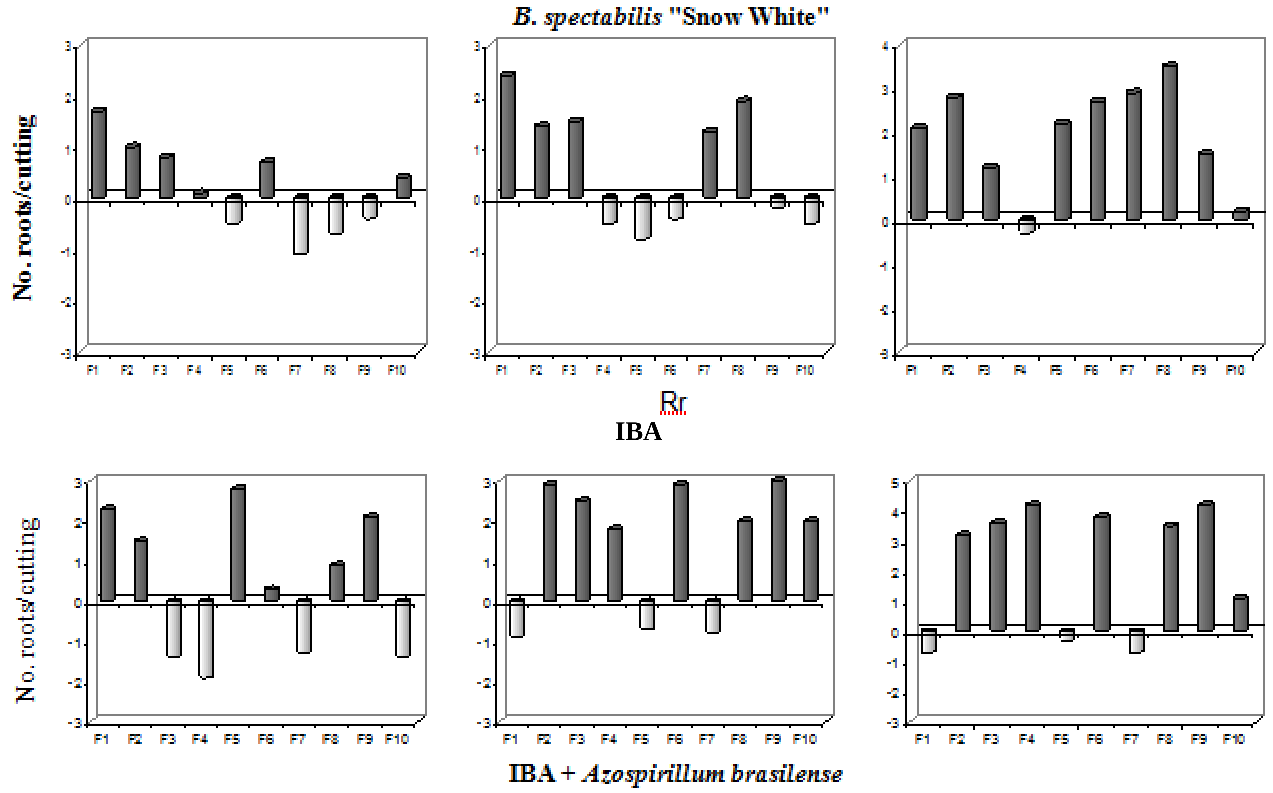
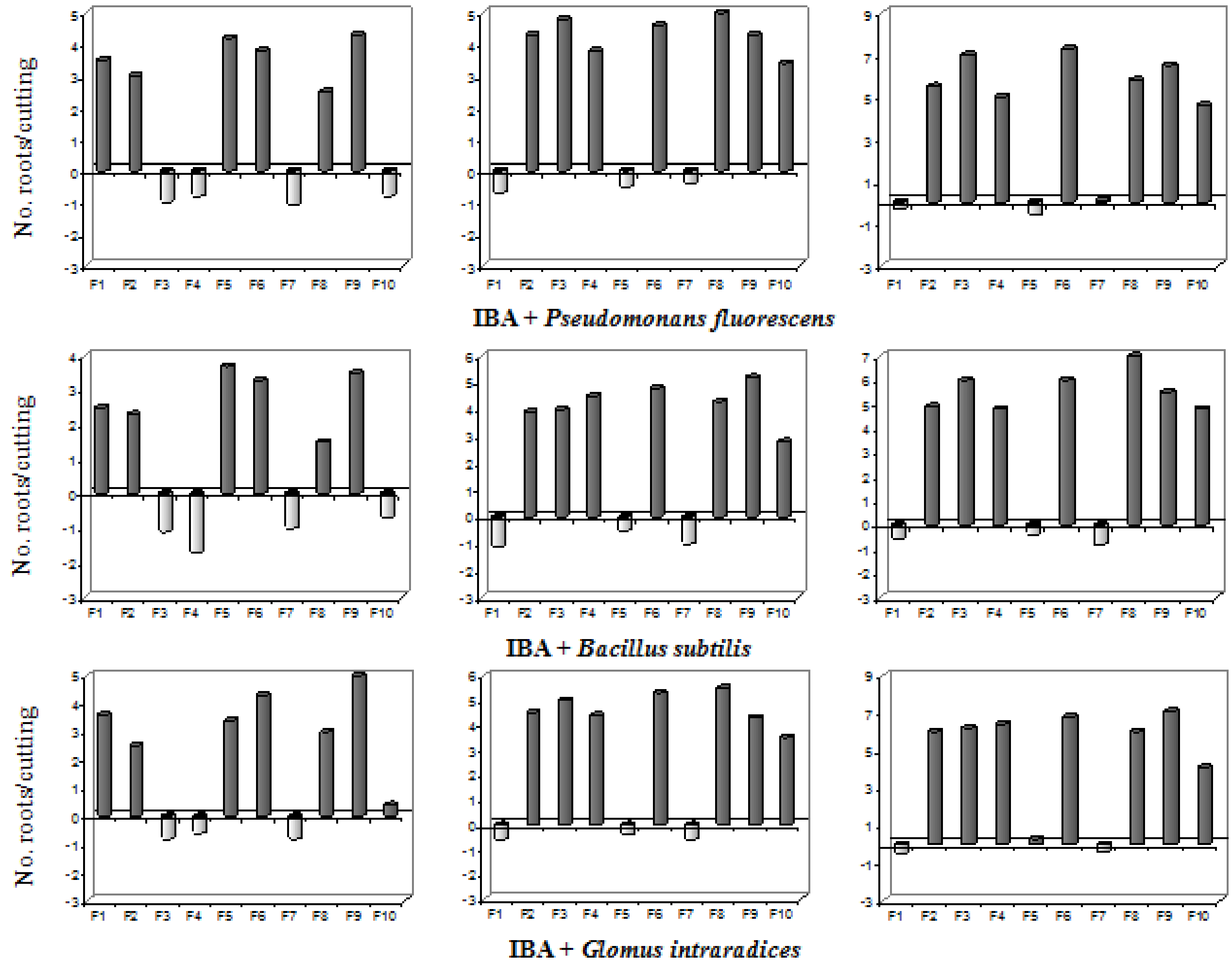


Figure 5: Effect of cutting extracts of the 4 *Bougainvillea* cultivars on mean number of roots per mung bean cutting as affected by branch portions.



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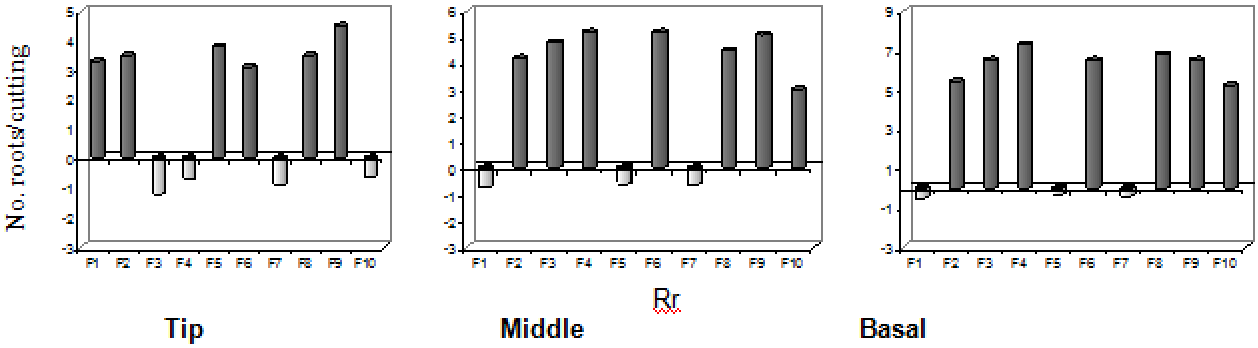

Figure 6: Effect of cutting extracts of the *B. glabra* var. *sanderiana* on mean number of roots per mung bean cutting as affected by PGPR and AMF

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