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Impact of IBA and bio-inoculants on growth and rhizogenesis in pomegranate (*Punica granatum*)

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ABSTRACT

The present experiment was conducted to study the effect of Indole butyric acid (IBA) as alone or in combination with synergetic bio-inoculants i.e. *Azotobacter*, phosphate solubilizing microorganisms (PSM) and Arbuscular Mycorrhizae (AM fungi) on rhizogenesis, vegetative growth parameters, biomass accumulation and nutrient uptake in hardwood cuttings of pomegranate (*Punica granatum* L.). Fifteen treatments excluding the control (water dipping) treated with IBA and inoculated with bio-inoculants alone or in different combinations were examined. All root and shoot related parameters were significantly improved in the cuttings treated with IBA and inoculated with bio-inoculants treatments. Maximum cuttings survival %, diameter of primary root, fresh and dry root weight, number of primary roots, plant height, number of branches, shoot diameter and shoots fresh weight was observed in cuttings treated with IBA and pre-inoculated with PSM + AM fungi followed by AM fungi + *Azotobacter* combinations. This study gives the future insight for exogenous applications of IBA with ecofriendly bio-inoculants in improving vegetative growth and increasing the production of healthy plants of horticultural crops under nursery conditions.

Key words: Bio-inoculants, Growth regulator, Nursery, Pomegranate

Pomegranate (*Punica granatum* L.) has gained a lot of importance, and interest of traditional medical practitioners, nutritionists, research scholars and consumers owing to its nutraceutical and therapeutical properties, presence of several bio-active compounds and potentially active antioxidants, viz. tannin, polyphenol, flavonoid and ascorbic acid (Elfalleh *et al.* 2012). India produces about 2.79 mt of pomegranate from an area of 2.20 lakh/ha (Anonymous 2018) and area under its cultivation has almost doubled and production has been three times for the last one decade. In pomegranate, application of IBA to the basal portion of mature shoot cuttings is remarkably more effective for root initiation, higher roots production, increment in shoot growth as well as dry weight for the both roots and shoots (Owais 2010).

Fruit nursery production globally has undergone significant changes in recent years due to the application of bio-inoculants for enhancing plant growth and fruit production. Plant growth promoting microbes act as catalysts in various mechanisms, viz. biological nitrogen fixation, production of growth hormones, phosphate solublization and production of many hydrolytic enzymes (Tailor and Joshi 2014). The synergistic role of bio-inoculants in improving the efficacy of applied nutrients has solved the problem of stagnation in productivity of perennial fruit crops (Srivastava *et al.* 2015). Application of bio-inoculants substantially improves the microbial activity in the rhizosphere and subsequently maintains the soil productivity and quality at low input costs. The collective effect of growth regulators and bio-inoculants considerably improved the root and shoot growth parameters in pomegranate as observed by Aseri *et al.* (2008). The present study, therefore, focused on the effect of IBA and bio-inoculants on survival %, vegetative growth and nutrient uptake of pomegranate. It was hypothesized that the application of IBA and bio-inoculants will have effect on rhizogenesis and plant growth of pomegranate under sub-tropical agro-climatic region of north India.

MATERIALS AND METHODS

Experimental site and treatments: The present study was conducted for two consecutive years (2016–17, 2017–18) at Fruit Research Farm, Punjab Agricultural University, Ludhiana located at 30.9°N latitude, 75.8° E longitude at 249 msl. The soil comprising 128 g clay, 151 g silt and 716 g sand/kg, *p*H 7.8 was free from salinity (0.33 dS/m). The soil had medium organic carbon 4.0 g OC/kg and 26.1 mg extractable P/kg and 320.7 mg NH_4OAc-extractable K/kg. The hardwood cuttings prepared in January were treated with IBA alone or with bio-inoculants, viz. IBA 200 ppm (T_1), IBA 200 ppm + *Azotobacter chroococcum* (T_2), *Azotobacter chroococcum* (T_3), IBA + Phosphate Solubilizing microorganisms (PSM) (T_4), PSM (T_5), IBA

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200 ppm + AM fungi (*Glomus mosseae*) (T_6), AM fungi (T_7), IBA 200 ppm + *Azotobacter chroococcum* +PSM (T_8), *Azotobacter chroococcum* + PSM (T_9), IBA + AM fungi + *Azotobacter chroococcum* (T_{10}), AM fungi + *Azotobacter chroococcum* (T_{11}), IBA+ AM fungi + PSM (T_{12}), AM fungi + PSM (T_{13}), IBA + *Azotobacter chroococcum* + PSM + AM fungi (T_{14}), *Azotobacter chroococcum* + PSM + AM fungi (T_{15}) and the control (water dipping) (T_{16}). These sixteen treatment combinations were replicated thrice in Randomized Block Design (RBD).

The viable culture of *Azotobacter chroococcum*, AM fungi (*Glomus mossae*) and phosphate solubilizing microorganisms (PSM) maintained at Department of Microbiology, Punjab Agricultural University, Ludhiana were used for the experimental purposes. The soil of experimental site was covered with transparent plastic sheet for 21 days for solarization to completely eliminate the soil borne pathogens. Farmyard manure (FYM) was mixed thoroughly in upper 30 cm soil in the ratio of 1:1 (v/v). FYM was mixed with nursery bed soil that contained 20.5% OC, 0.42% P_2O_5 and 0.45% K_2O. Hardwood cuttings of pomegranate cv. *Bhagwa* of uniform size (20–30 cm length and 1–1.25 cm diameter) with about 4-5 buds were prepared from well-established healthy mother block during 1st fortnight of January. The required quantity of Indole butyric acid (200 ppm) was dissolved in ethanol solution and few drops of NH_4OH were added to avoid precipitation. The final volume was prepared in deionized water. Treatments, viz. T_1, T_2, T_4, T_6, T_8, T_{10}, T_{12} and T_{14} were treated with IBA solution for 24 h and subsequently dried under shade. IBA treated and untreated cuttings (except control) were dipped in respective bio-inoculant culture (100 g/l of water) for 30 min and treatments were replicated thrice. These were transplanted on nursery beds of dimension 2.5 m × 1.0 m spaced at 30 cm × 30 cm. Uniform cultural practices were followed during the study period.

Plant analysis: Data on root and shoot growth characters and dry weight were estimated after one year (dormant stage) when the sprouted cuttings were ready for transplantation. For leaf nutrient analysis, nearly 30–40 fully opened leaves were collected as per the procedure described by Bhargava and Chadha (1988). Leaves were disinfected using 0.2% Teepol solution and 0.1 N HCl; and then washed twice with deionized water. Leaf samples were dried in hot air oven at 65 ± 1°C until a stage of constant weight was achieved. Oven dried samples were ground and sieved through 1 mm mesh size and analyzed for N, P and K content as per standard procedures.

Statistical analysis: Data of both the years under study was pooled and subjected to analysis of variance using SAS (version 9.3, SAS institute Inc, USA,). The interactions found significant were subjected to mean separation using LSD ($P \leq 0.05$).

RESULTS AND DISCUSSION

Survival %: The results (Fig 1) revealed that survival of pomegranate hardwood cuttings ranged from 65.3–81.7% in all the treatments except the control (55.5). Treatments IBA + PSM, IBA + Azotobacter + PSM, IBA + AM fungi + Azotobacter, IBA + AM fungi + PSM and IBA + Azotobacter + PSM + AM fungi showed more than 80% survival after one year of inoculation treatments. Maximum survival % was displayed by the cuttings treated with IBA + AM fungi + PSM, which was statistically at par with IBA + AM fungi + *Azotobacter*, IBA + *Azotobacter* + PSM and IBA + PSM. These results are in accordance with the findings of Karakurt *et al.* (2009). Increased growth with application of IBA combined with bio-inoculants may be attributed to their ability to synthesize natural growth promoter IAA and considerably increased uptake of N and K elements due to

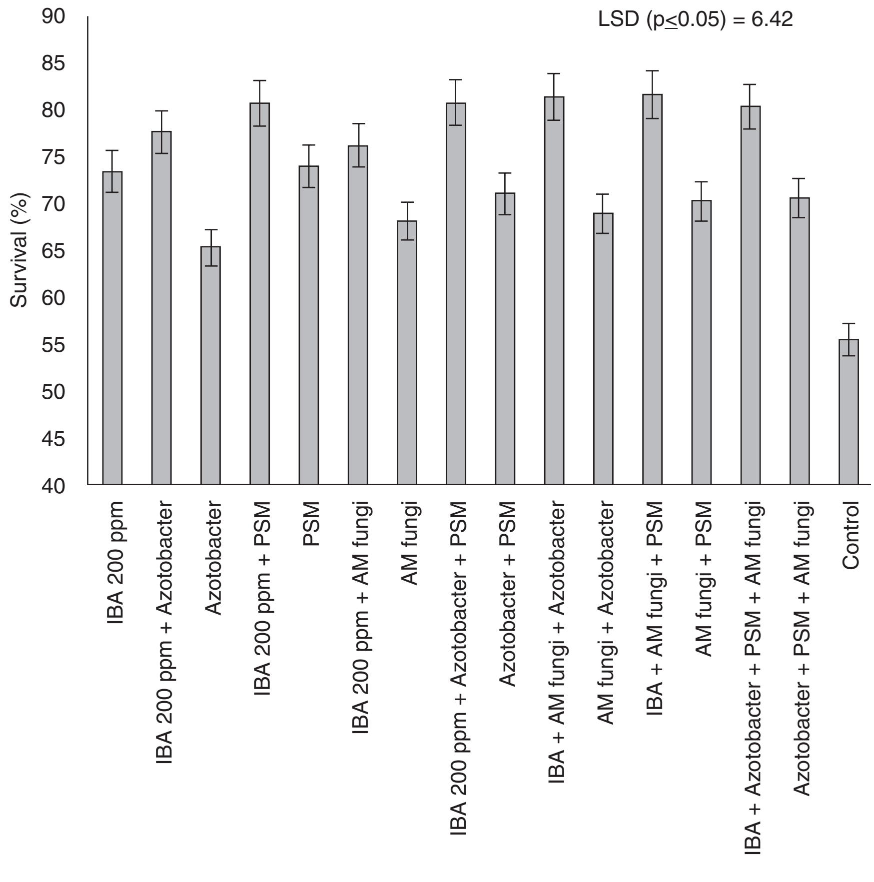

Fig 1 Effect of IBA and bio-inoculants on survival (%) in cuttings of pomegranate cv. *Bhagwa*

Table 1 Effect of IBA and bio-inoculants on root characters in cuttings of pomegranate cv. *Bhagwa*

Treatment	Root length (cm)	Diameter of root (mm)	Number of roots	Root fresh weight (g)	Root dry weight (g)
IBA 200 ppm	25.04 ± 0.36^{fg}	3.08 ± 0.07^{ef}	10.00 ± 0.13^{c}	5.33 ± 0.10^{f}	2.12 ± 0.03^{fg}
IBA 200 ppm + *Azotobacter*	26.13 ± 0.38^{ef}	3.29 ± 0.07^{cd}	10.12 ± 0.13^{bc}	5.70 ± 0.11^{de}	2.25 ± 0.03^{de}
Azotobacter	23.13 ± 0.34^{h}	2.85 ± 0.06^{g}	7.50 ± 0.10^{g}	4.30 ± 0.08^{ij}	1.68 ± 0.02^{i}
IBA 200 ppm + PSM	26.96 ± 0.39^{de}	3.41 ± 0.08^{c}	8.10 ± 0.10^{ef}	5.93 ± 0.12^{cd}	2.35 ± 0.03^{c}
PSM	25.69 ± 0.37^{f}	2.92 ± 0.07^{fg}	7.50 ± 0.10^{g}	5.00 ± 0.10^{gh}	1.95 ± 0.02^{h}
IBA 200 ppm + AM fungi	27.12 ± 0.39^{de}	3.18 ± 0.07^{de}	10.30 ± 0.13^{abc}	5.85 ± 0.12^{d}	2.30 ± 0.03^{cd}
AM fungi	24.41 ± 0.35^{g}	2.79 ± 0.06^{g}	7.33 ± 0.09^{g}	4.43 ± 0.09^{i}	1.74 ± 0.02^{i}
IBA 200 ppm + *Azotobacter* + PSM	28.24 ± 0.41^{bc}	3.65 ± 0.08^{ab}	10.40 ± 0.13^{ab}	6.25 ± 0.12^{bc}	2.47 ± 0.03^{b}
Azotobacter + PSM	27.39 ± 0.40^{cd}	2.78 ± 0.06^{g}	7.90 ± 0.10^{f}	4.95 ± 0.10^{gh}	1.95 ± 0.02^{h}
IBA+ AM fungi + *Azotobacter*	30.20 ± 0.44^{a}	3.48 ± 0.08^{bc}	10.50 ± 0.14^{a}	6.48 ± 0.13^{ab}	2.55 ± 0.03^{ab}
AM fungi + *Azotobacter*	24.37 ± 0.35^{g}	3.12 ± 0.07^{def}	7.40 ± 0.09^{g}	4.76 ± 0.09^{h}	1.86 ± 0.02^{h}
IBA + AM fungi + PSM	29.97 ± 0.43^{a}	3.71 ± 0.08^{a}	10.51 ± 0.14^{a}	6.71 ± 0.13^{a}	2.64 ± 0.03^{a}
AM fungi + PSM	25.56 ± 0.37^{f}	2.98 ± 0.07^{efg}	8.30 ± 0.11^{e}	5.18 ± 0.10^{fg}	2.06 ± 0.03^{g}
IBA + *Azotobacter* + PSM + AM fungi	28.57 ± 0.42^{b}	3.49 ± 0.08^{bc}	10.33 ± 0.13^{abc}	6.32 ± 0.12^{b}	2.47 ± 0.03^{b}
Azotobacter + PSM + AM fungi	25.59 ± 0.37^{f}	3.17 ± 0.07^{de}	9.20 ± 0.12^{d}	5.50 ± 0.10^{ef}	2.16 ± 0.03^{ef}
Control	19.90 ± 0.29^{i}	2.44 ± 0.06^{h}	7.30 ± 0.09^{g}	4.08 ± 0.08^{j}	1.55 ± 0.02^{j}
LSD ($P \leq 0.05$)	1.11	0.20	0.35	0.32	0.08

Means with same letter are not significantly different at $P \leq 0.05$

Table 2 Effect of IBA and bio-inoculants on shoot growth traits in cuttings of Pomegranate cv. *Bhagwa*

Treatment	Plant height (cm)	Shoot diameter (mm)	No. of branches	Number of leaves /plant	Fresh weight of shoots (g)	Dry weight of shoots (g)
IBA 200 ppm	87.467 ± 2.28^{g}	9.37 ± 0.27^{abcde}	8.20 ± 0.21^{de}	39.10 ± 0.88^{efg}	129.10 ± 2.17^{gh}	68.16 ± 1.89^{ef}
IBA 200 ppm + *Azotobacter*	104.48 ± 2.72^{abcd}	9.66 ± 0.28^{abcd}	8.20 ± 0.21^{de}	40.20 ± 0.91^{def}	147.90 ± 2.48^{cde}	77.71 ± 2.15^{c}
Azotobacter	97.76 ± 2.73^{def}	8.71 ± 0.28^{ef}	8.00 ± 0.21^{de}	38.60 ± 0.91^{fg}	128.0 ± 2.48^{gh}	68.94 ± 2.16^{e}
IBA 200 ppm + PSM	99.75 ± 2.60^{de}	9.92 ± 0.28^{ab}	8.90 ± 0.23^{ab}	42.00 ± 0.95^{bcd}	153.80 ± 2.59^{bc}	81.53 ± 2.26^{bc}
PSM	101.01 ± 2.64^{cde}	9.05 ± 0.26^{cdef}	7.90 ± 0.20^{de}	40.90 ± 0.92^{bcdef}	143.0 ± 2.40^{ef}	76.19 ± 2.12^{cd}
IBA 200 ppm + AM fungi	97.23 ± 2.53^{def}	9.37 ± 0.27^{abcde}	8.30 ± 0.21^{cd}	37.40 ± 0.84^{gh}	145.40 ± 2.44^{def}	76.63 ± 2.13^{c}
AM fungi	88.1 ± 2.30^{g}	8.72 ± 0.25^{ef}	8.00 ± 0.20^{de}	37.50 ± 0.85^{gh}	122.60 ± 2.06^{h}	62.78 ± 1.74^{fg}
IBA 200 ppm+ *Azotobacter* + PSM	103.2 ± 2.69^{bcd}	9.59 ± 0.27^{abcd}	8.80 ± 0.22^{abc}	43.00 ± 0.97^{abc}	150.60 ± 2.53^{cd}	81.17 ± 2.25^{bc}
Azotobacter + PSM	95.55 ± 2.49^{ef}	8.94 ± 0.26^{def}	8.10 ± 0.20^{de}	43.10 ± 0.98^{abc}	131.70 ± 2.21^{g}	69.25 ± 1.92^{e}
IBA+ AM fungi + *Azotobacter*	107.52 ± 2.81^{abc}	9.59 ± 0.27^{abcd}	8.00 ± 0.20^{de}	41.70 ± 0.94^{bcde}	150.8 ± 2.53^{cd}	80.68 ± 2.24^{bc}
AM fungi + *Azotobacter*	91.35 ± 2.38^{fg}	8.94 ± 0.26^{def}	8.40 ± 0.21^{bcd}	40.60 ± 0.92^{cdef}	113.5 ± 1.91^{i}	61.09 ± 1.69^{g}
IBA + AM fungi + PSM	111.09 ± 2.90^{a}	10.03 ± 0.29^{a}	9.00 ± 0.23^{a}	43.30 ± 0.98 ^{ab}	163.3 ± 2.74^{a}	89 ± 2.47^{a}
AM fungi + PSM	99.33 ± 2.59^{de}	9.16 ± 0.26^{bcdef}	7.70 ± 0.19^{e}	39.20 ± 0.88^{efg}	140.2 ± 2.35^{f}	75. 89 ± 2.10^{cd}
IBA + *Azotobacter* + PSM + AM fungi	109.83 ± 2.86^{ab}	9.81 ± 0.28^{abc}	8.30 ± 0.21^{cd}	45.10 ± 1.02 ^{a}	158.10 ± 2.66^{ab}	86.32 ± 2.40^{ab}
Azotobacter + PSM + AM fungi	99.44 ± 2.59^{de}	9.59 ± 0.27^{abcd}	8.90 ± 0.23^{ab}	41.30 ± 0.93^{bcde}	132.70 ± 1.96^{g}	70.45 ± 1.96^{de}
Control	77.60 ± 2.02^{h}	8.50 ± 0.24^{f}	7.70 ± 0.19^{e}	35.80 ± 0.81 ^{h}	93.4 ± 1.57^{j}	46.14 ± 1.28^{h}
LSD ($P \leq 0.05$)	7.40	0.76	0.59	2.64	6.71	5.91

Means with same letter are not significantly different at $P \leq 0.05$

biological nitrogen fixation and phosphate solubilization. Prolific root initiation and better cuttings survival % was also reported with the application of auxins due to synergistic interactions with bio-inoculant species (Niemi *et al.* 2002).

Root parameters: Cuttings treated with IBA + *Azotobacter* + AM fungi significantly had the longest (30.20 cm) root length which was at par with 29.97 cm with IBA + AM fungi + PSM, followed by 28.57 cm in IBA + *Azotobacter* + PSM + AM Fungi (Table 1). *Azotobacter*, AM fungi, PSM and IBA (200 ppm) alone substantially registered increment in root length to the tune of 16.2, 22.7, 29.1 and 25.8%, respectively. IBA + either of the dual combinations of PSM, AM Fungi and *Azotobacter* also enhanced the length of primary roots by about 16.2–51.8% over the untreated. However, maximum root diameter (3.71 mm) was noted in the cuttings treated with IBA + AM fungi + PSM. These results corroborate with the findings of Karakurt *et al.* (2009). Improvement in root length and diameter may be attributed to the early initiation of roots with the application of IBA and also supported by higher nutrients uptake and biomass accumulation due to dipping of cuttings in bio-inoculants solution (Hakim *et al.* 2018).

Significantly higher number of primary roots/plant were obtained in the plants treated with IBA and bio-inoculants (Table 1). It was observed to be maximum (10.51) in the cuttings treated with IBA + AM fungi + PSM, which was statistically at par with IBA + AM fungi + Azotobacter (10.50), accounting to nearly 44% over the control as reported earlier also by Ansari (2013). Fresh root weight was maximum (6.71 g) in IBA + AM fungi + PSM. Similarly, significantly higher (2.64 g) dry root weight was recorded in IBA + AM fungi + PSM as compared to other treatments where it ranged from 1.68 g in *Azotobacter* treated cuttings to 2.55 g in IBA + AM fungi +*Azotobacter* treatment, as compared to the control (1.55 g). Landmark study of Pacurar *et al.* (2014) offers valuable insights regarding the role of auxins in adventitious root formation.

Shoot growth parameters: The defined scrutiny of the data revealed that maximum plant height of 111.09 cm was recorded in the cuttings treated with IBA + AM fungi + PSM which accounted for increment to the tune of 43.2% over the control (Table 2), closely followed by IBA + AM fungi + *Azotobacter* + PSM (109.83 cm). IBA + AM fungi + PSM proved to be best for the production of primary branches/plant (9.0) with diameter 10.03 mm. Similar improvement in shoot characters with the application of IBA has been reported by Dhillon and Sharma (1992) in pomegranate cuttings under north Indian conditions.

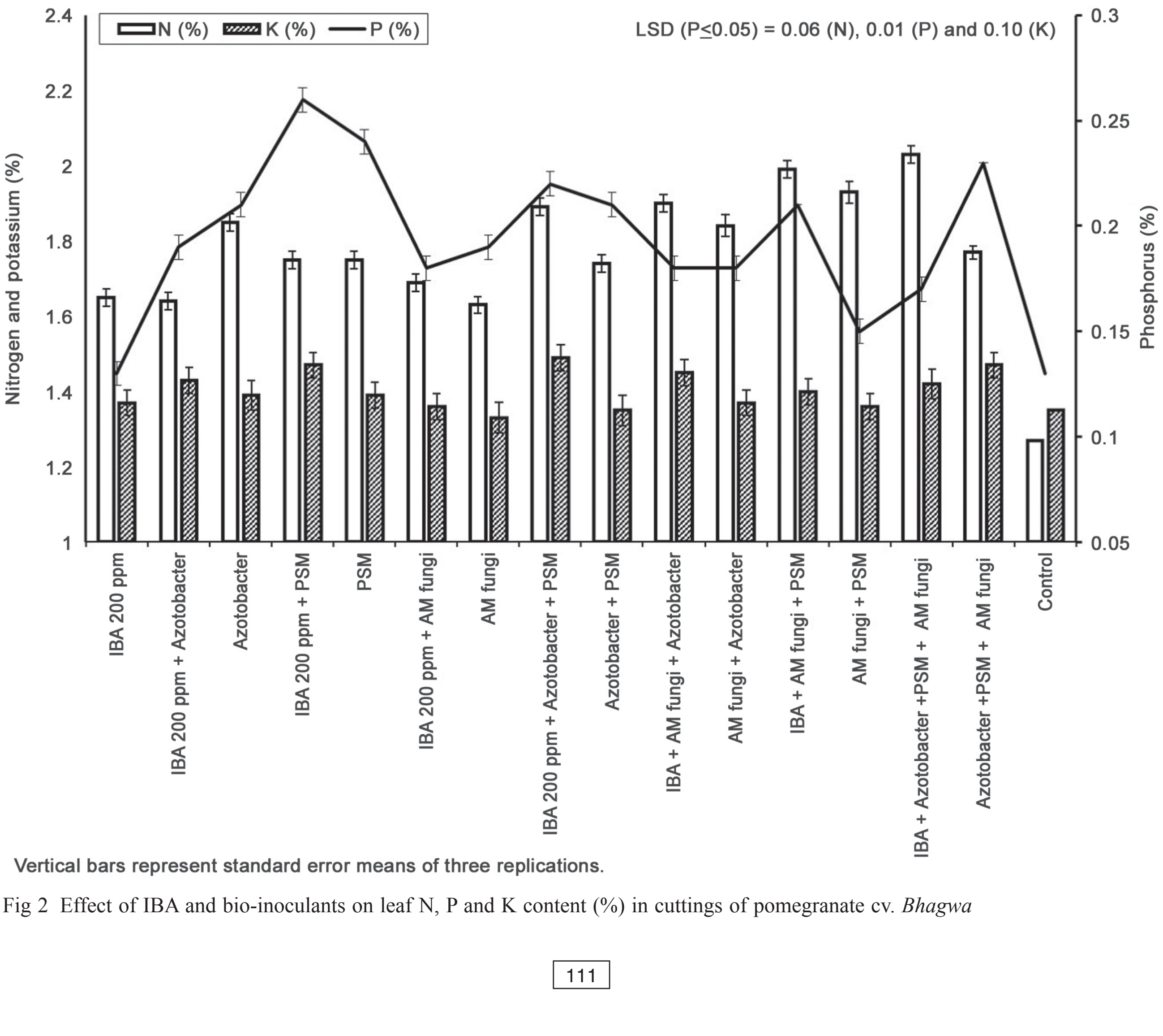

Fig 2 Effect of IBA and bio-inoculants on leaf N, P and K content (%) in cuttings of pomegranate cv. *Bhagwa*

Pre-inoculated pomegranate hardwood cuttings with bio-inoculants may have maximum survivability due to the production of vigorous roots and shoot system and higher biomass production than single or dual bio-inoculant treatment without IBA.

A treatment combination of IBA + AM fungi + *Azotobacter* + PSM had maximum number of leaves/plant (45.10) than the control (35.80 leaves/plant) (Table 2). The exogenous application of IBA primarily upsurges cell division and cell elongation, and greatly improved shoot growth (Seiar 2017). Shoot fresh and dry weight also showed similar trend. Improvement in cell division, cell elongation, growth of vigorous root and shoot systems in hardwood cuttings of pomegranate was due to higher natural auxin biosynthesis which was further boosted by the synergistic effect of bio-inoculants, as reported by Kumar *et al.* (2016) in pomegranate.

Leaf N, P and K content: Leaf N, P and K levels in all the treatments (Fig 2) were within optimum range (0.44–2.54, 0.10–0.26 and 0.20–2.37%, respectively) after one year of treatment (Raghupati and Bhargava 1998). Leaf N content was significantly highest (2.03%) in the cuttings treated with IBA + *Azotobacter* + PSM + AM fungi, which statistically was at par with IBA + AM fungi + PSM (1.99%). Minimum leaf N content of 1.27% was registered in the control. Among all treatments, P accumulation was found maximum in the treatments having PSM as a constituent, as compared to other bio-inoculant applications. Potassium content did not show any specific trend with different treatment combinations and the difference between the most of the treatments was statistically non-significant. Leaf P content accumulation in the cuttings treated with PSM alone or in combination with other bio-inoculations might be due to the presence of microorganisms are known to have the ability to solubilize inorganic P from inorganic sources, thus making it readily available to the plants (Venkateswarlu *et al.* 2008).

The present investigation suggests positive response of growth regulators and bio-inoculants on survivability and growth of hardwood cuttings of pomegranate in nursery which may be helpful in establishment of healthy plantation for sustainable production.

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