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Effect of *Azotobacter* and *Sphingobacterium* species on guava seedlings under nursery conditions

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ABSTRACT

An experiment was planned to study the effect of biofertilizers, namely *Azotobacter* and *Sphingobacterium* species on guava seedlings under nursery conditions. The standard culture of *Azotobacter* and *Sphingobacterium* species was obtained from Department of Microbiology, Punjab Agricultural University, Ludhiana, Punjab, India. The liquid formulations of microbial inoculants were prepared by supplementing 2% PEG in basal medium. The three months old guava seedlings were given dip treatment for 30 mins with liquid microbial inoculants and transplanted in nursery bed and observations were recorded after one year. The treated guava seedlings showed very good response to *Azotobacter* and *Sphingobacterium* species treatment (T_3) followed by *Azotobacter* sp. (T_2). Seedlings inoculated with *Azotobacter* and *Sphingobacterium* species inoculation showed an increase of 4.28 per cent in shoot length, 4.41 per cent in collar diameter, 14.55 per cent in root length, 3.85 per cent in root numbers, 9.33 per cent in number of main branches/plant, 7.95 per cent in number of leaves, 6.95 per cent in fresh weight of shoot, 8.27 per cent in dry weight of shoot, 15.08 per cent in fresh leaf weight, 14.08 per cent in dry weight of leaves, 12.60 per cent in fresh weight of root and 12.69 per cent in dry weight of root over control. The use of biofertilizer offers better options for enhancing the vegetative growth of horticultural crops under nursery conditions in an increasingly eco-conscious world, thus increasing the success rate of vegetative propagation in healthy plants with well developed root and shoot system.

Key words: *Azotobacter*, *Sphingobacterium*, Bio-fertilizers.

INTRODUCTION

Guava is an important fruit of tropical and subtropical area of the world. It is commonly called poor man's fruit. Guava contains maximum vitamin C content per l00g of pulp after amla. It contains antioxidant factors and can control systolic blood pressure. It is good source of roughage and help in removal of constipation. In India, area under guava during the year 1987-88 was 176.8 thousand hectares, which has increased to 234.06 thousand hectares during the year 2011-12. India has made a fairly good progress in production from the year 1987-88 to 2011-12. It increased from 1112.6 thousand tonnes to 2660.76 thousand tonnes. The productivity of guava has increased from 6.3 tonnes to 11.70 tonnes during above period (Kumbhar *et al*., 9). One of the most important factor contributing towards high productivity of fruit crops is quality planting materials. Shortage of planting material is a major problem in the production of horticultural crops. There is an immense scope of employment and income generation through production and supply of quality planting material in horticultural crops.

Perennial fruit crops are heavy feeders of plant nutrients and high yields can only be sustained through the application of optimal doses in balanced proportion. Nutrient management is one of the largest shares of cost with its impact on potential yield and crop quality (Ganeshamurthy *et al*., 7). Chemical fertilizers today are an indispensible part of modern orchard practices (Bala *et al*., 3). Continuous use of chemical fertilization leads to the deterioration of soil health and productivity. In this context, biofertilizers have emerged as an important component of the integrated nutrient supply system and have great potential to improve crop yields through environmentally better nutrient supplies (Das *et al*., 5). They are known to improve fixation of nutrients in the rhizosphere, produce growth stimulants for plants, improve soil stability, provide biological control, biodegrade substances, recycle nutrients, promote mycorrhiza symbiosis and develop bioremediation processes in soils contaminated with toxic, xenobiotic and recalcitrant substances (Rivera-Cruz *et al*., 12). Biofertilizers keep the soil environment rich in all kinds of micro- and macro-nutrients via nitrogen fixation, phosphate and potassium solubilisation or mineralization, release of plant growth regulating substances, production of antibiotics and biodegradation of organic matter in the soil (Sinha *et al*., 13) providing better nutrient uptake and increased tolerance towards drought and moisture stress. Biofertilizers differ from chemical

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and organic fertilizers in the sense that they do not directly supply any nutrients to crops and are cultures of special bacteria and fungi, relatively simple and having low installation cost (Alam and Seth, 1).

In today's scenario there is an increasing demand of horticultural crops in India. To meet this demand quality planting material is prerequisite. Thus nursery business is flourishing at a fast pace. Keeping in view the economic, environmental and agronomic importance of biofertilizers an experiment was planned for the production of quality planting material for horticultural crop i.e guava. The present experiment was carried out to study the effect of *Azotobacter* sp. and *Sphingobacterium* sp. on vegetative growth parameters of guava under nursery conditions for the development of healthy planting material with well developed shoot and root system for vegetative propagation.

MATERIALS AND METHODS

The study has been conducted to assess the influence of *Azotobacter* and *sphingobacterium* species on vegetative growth and biomass accumulation of guava seedlings under nursery conditions for the production of quality planting material. The standard culture of *Azotobacter* and *Sphingobacterium* species was obtained from the Department of Microbiology, PAU, Ludhiana. Liquid microbial inoculants of individual cultures were prepared by supplementing 2% Polyethylene Glycol (PEG) in basal medium (NaCl 5g/L, Glucose 10g/L, Yeast Extract 3.0g/L). It has a shelf life of three month at ambient temperature and used @ 250ml/acre respectively (liquid microbial inoculants, $1x10^8$ colony forming unit (CFU) per ml). The liquid inoculants for one acre can be diluted in 10-15 liters of water and use accordingly for dip treatment. Both the cultures used in this study are positive for IAA production and Phosphate solubilization. In addition, *Azotobacter* sp. is also positive for NH_3 production. The three month old guava seedlings of uniform vigour and height were collected (10-12 cm of height). The dwarf and bigger seedlings were discarded and the roots of selected seedlings were given dip treatment by dipping in respected liquid biofertilizer culture for 30 mins and transplanted on to the nursery beds of size 2X1m in rows of 15 cm apart. The data on vegetative growth parameters in terms of shoot length (cm), collar diameter (cm), root length (cm), root numbers, number of main branches/ plant, number of leaves and biomass accumulation in terms of fresh and dry weight of shoot (g), fresh and dry weight of leaves (g) and fresh weight and dry weight of roots (g) were recorded after one year of transplanting. There were three treatments, T_1: Control, T_2: *Azotobacter* sp., T_3: *Azotobacter* sp. and *Sphingobacterium* sp. replicated four times with 50 plants/ replication in a randomised block design. Analysis of variance (ANOVA) and the test of mean comparison according to critical difference (CD) were applied. Significance level was accepted at $p \leq 0.05$. The data was analyzed statistically by randomized block design using CPCS1 software as a statistical analysis tool (Cheema and Singh, 4).

RESULTS AND DISCUSSION

Inoculation with microbial inoculants had a significant ($p \leq 0.05$) effect on growth parameters of guava seedlings. The shoot length (125.80 cm) and collar diameter (1.42 cm) was recorded significantly ($p \leq 0.05$) higher from the seedlings treated with *Azotobacter* sp. and *Spingobacterium* sp. (T_3) accounting to about 4.28 per cent increase in shoot length and 4.41 per cent increase in collar diameter over control (T_1). This was followed by treatment with *Azotobacter* sp. (T_2) with 2.48 per cent increase in shoot length and 2.94 per cent increase in collar diameter with respect to control (T_1). An increase in plant height and spread with the application of biofertilizers in guava was also reported by Dutta *et al*. (6). It may be due to enhanced N and P availability to the plant. Apart from its ability to fix atmospheric nitrogen, *Azotobacter* sp. used in this study also synthesize biologically active growth substance such as indole acetic acid whereas *Sphingobacterium* sp. has the ability to solubilize inorganic P from insoluble sources and make available fixed forms of soil P. These properties of the two biofertilizers seemed to have enhanced the availability of both the nutrients (N and P) and benefit the plant. An increase in plant growth might also be due to the improvement in physio-chemical properties of soil; increase in enzymatic activity and microbial population by application of microbial inoculants.

Root length and number of roots of guava plants varied significantly ($p \leq 0.05$) with biofertilizers inoculation. The significantly higher root length of 37.80 cm was recorded from the plants treated with *Azotobacter* and *Sphingobacterium* species (T_3) accounting to about 14.55 per cent increase over control (33.00 cm) (T_1) followed by the treatment with *Azotobacter* sp. (T_2) with 35.88 cm root length. Similarly number of roots also get improved due to dual inoculation of microbial inoculants viz. *Azotobacter* and *Sphingobacterium* species (T_3) accounting to about 3.85 per cent increase over control (35.05 cm) (T_1). An increase in root length due to application of *Azotobacter* and *Sphingobacterium* species could be attributed to their capability to

synthesize biologically active substances like IAA and increased uptake of essential macronutrients like nitrogen and phosphorus due to biological nitrogen fixation and phosphate solubilization. Glick *et al.* (8) also reported that bacterial IAA increases root surface area and length, and thereby provides the plant greater access to soil nutrients. Also, rhizobacterial IAA loosens plant wall and as a result facilitates an increasing amount of root exudation that provides additional nutrients to support the growth of rhizophere bacteria. Rapid establishment of roots is advantageous for young seedlings as it increases their ability to anchor themselves to the soil and to obtain water and nutrients from their environment, therefore enhancing their chances for survival (Subramanian and Satyan, 14).

A significantly (p≤0.05) higher number of branches per plant (8.20) was reported from the plants treated with *Azotobacter* and *Sphingobacterium* species (T_3). An increase in number of branches was 9.33 per cent over the control (T_1). This was followed by the treatment with *Azotobacter* sp. (T_2) with 6.67 per cent (8.00 branches/plant) increase in number of branches over control i.e 7.50 branches/plant (T_1). The increased number of branches might be due to increased number of vegetative buds produced by taller plants. This is attributed to the ability of *Azotobacter* sp. to release IAA, solubilise phosphorus and fix nitrogen (Fig. 1) and phosphate solubilising activity and IAA producing potential of *Sphingobacterium* sp. The number of leaves varied significantly (p≤ 0.05) due to microbial inoculants. The maximum number of leaves per plant (133) was recorded from the plants treated with *Azotobacter* and *Sphingobacterium* species. (T_3) accounting to about 7.95 per cent increase over control (123.20) (T_1) followed by the treatment with *Azotobacter* sp. (T_2) with 129.50 number of leaves per plant. This could also be due to the production of IAA by *Azotobacter* and *Sphingobacterium* species. Naeem *et al.* (11) also reported that the application of IAA increased germination percentage, plant height, number of branches and leaves, total chlorophyll content and dry weight in *Lens culinaris*. IAA exerts influence on plant growth by enlarging leaves and increasing photosynthetic activities in plants. It also activates the translocation of carbohydrates during their synthesis (Awan *et al*., 2).

Biomass can be treated as true indicator of growth. Biomass content of the seedlings treated with biofertilizers inoculants varied significantly (p≤ 0.05). The fresh above the ground biomass (fresh shoot weight and fresh leaf weight) was recorded significantly (p≤ 0.05) higher from the plants treated with *Azotobacter* and *Sphingobacterium* species (T_3) followed by the treatment with *Azotobacter* sp. (T_2) (Fig. 2). An increase in 6.95 per cent in fresh weight of shoot and 15.08 per cent in fresh leaf weight was obtained in plants treated with *Azotobacter* and *Sphingobacterium* species (T_3) over control. If we

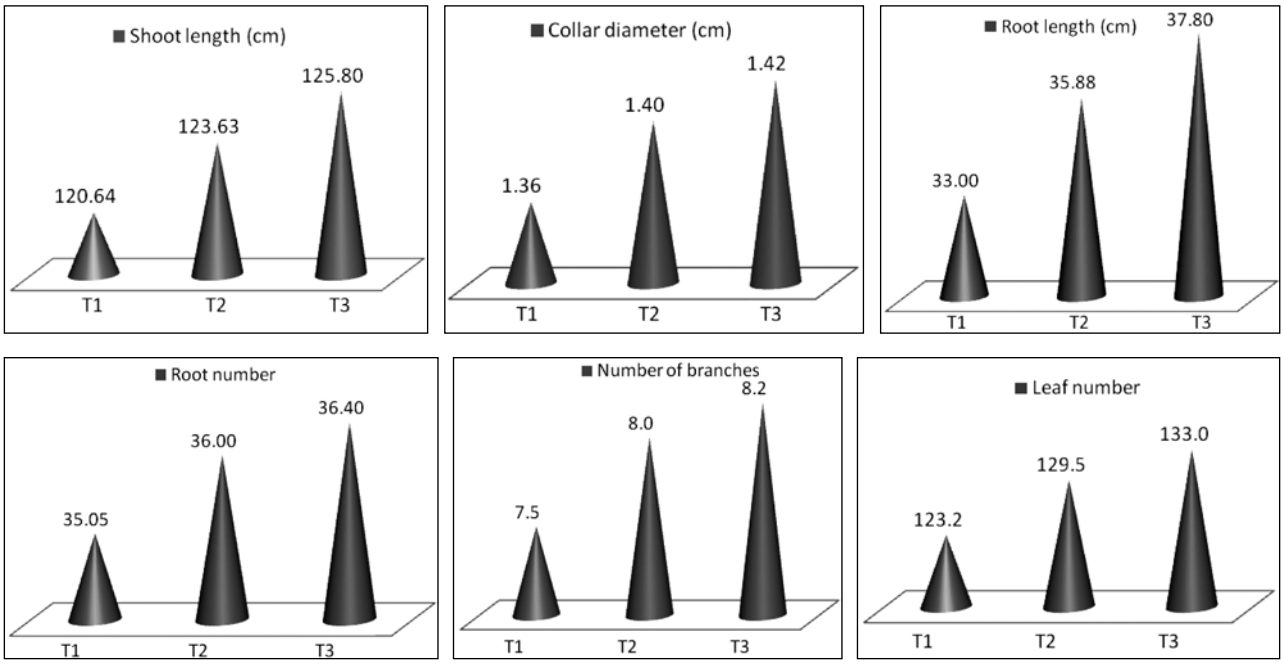

Fig. 1. Effect of microbial inoculants on shoot length, collar diameter, root length, root number, number of branches and leaf number of guava seedlings under nursery conditions.

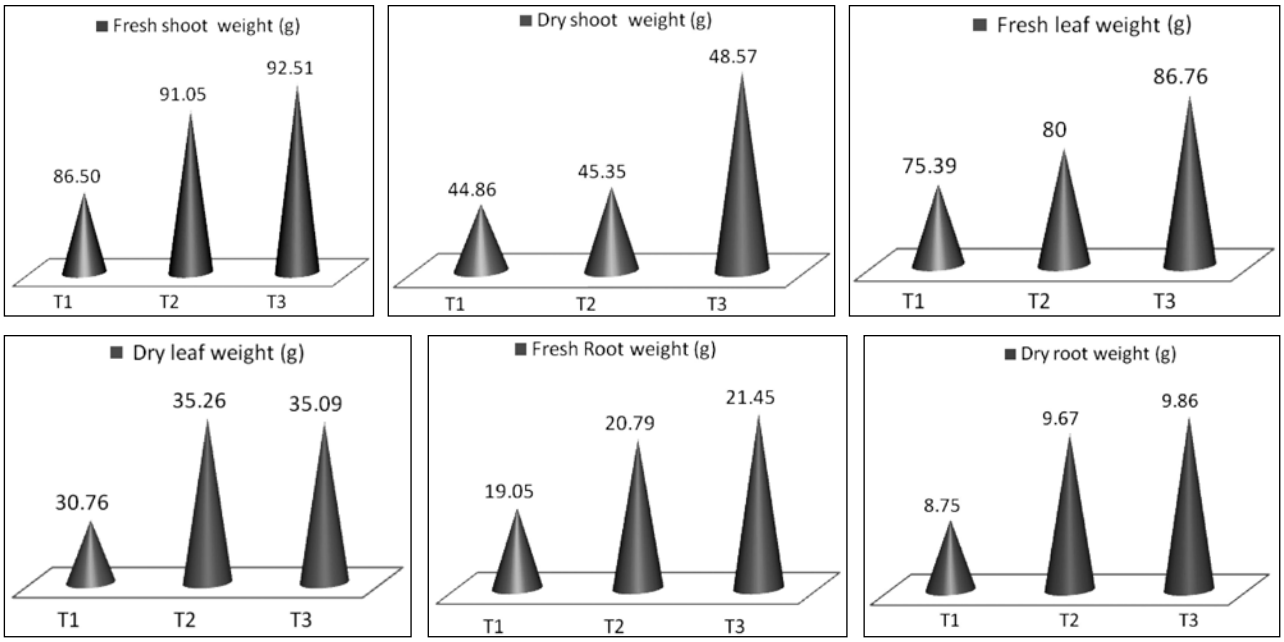

Fig. 2. Effect of microbial inoculants on biomass accumulation of guava seedlings under nursery conditions.

consider dry weight of shoot and leaves (above the ground biomass) again the best performance was given by T_3 (48.57 gm/plant and 35.26 gm/plant respectively) followed by T_2 (45.35 gm/plant and 35.09 gm/plant). An increase in 8.27 per cent in dry weight of shoot and 14.08 per cent in dry weight of leaves was recorded in plants treated with *Azotobacter* and *Sphingobacterium* species (T_3) over control. The fresh root biomass was found maximum in T_3 (21.45 gm/plant), followed by T_2 (20.79 gm /plant) and T_1 (19.05 gm/plant). For dry root biomass, T_3 (9.86 gm/plant) was the best treatment, followed by T_2 (9.67 gm/plant) and T_1 (8.75 gm/plant). The fresh weight of root was 12.60 per cent higher and dry weight of root was 12.69 per cent higher in plants treated with *Azotobacter* and *Sphingobacterium* species over control.

An overall increase in biomass accumulation by application of biofertilizer i.e *Azotobacter* and *Sphingobacterium* species may be due to the nitrogen fixing and phosphate solubilising activities of inoculated biofertilizer. In addition both the cultures (*Azotobacter* and *Sphingobacterium* species) used in this study produced indole acetic acid growth hormone. This hormone stimulates root growth and development. The use of growth stimulating inoculants helps to accelerate uptake of plant nutrients from applied chemical fertilizers by increasing the root growth. Thus, continuous use of bio-fertilizers can enables the microbial population to remain and build up in the soil and helps in maintaining soil fertility contributing to sustainable agriculture (Malik *et al*., 10).

CONCLUSION

The most prominent findings emerged was regarding superiority of seedling bacterization with *Azotobacter* and *Sphingobacterium* species over un inoculated control plants in terms of vegetative growth parameters and biomass accumulation due to atmospheric nitrogen fixing ability of *Azotobacter* sp. and Phosphate solubilizing activity of *Azotobacter* and *Sphingobacterium* species apart from their ability to produce IAA.

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