The Pharma Innovation Journal 2022; 11(6): 2077-2081

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(6): 2077-2081

www.thepharmajournal.com
Received: 22-03-2022
Accepted: 27-05-2022

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Effect of biofertilizers on seedling vigour of sweet leaf- *Stevia rebaudiana* Bertoni

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Abstract
The present investigation entitled, "Effect of Biofertilizers on Seedling Vigour of Sweet leaf- *Stevia rebaudiana* Bertoni" was carried out in experimental farm, located at Nauni of the Department of Forest Products. The experiment was laid out in completely randomized block design with eight treatments (T1 (Basal media (Sand: Soil: FYM:: 1:1:1), T2 (Basal media + Azotobacter), T3 (Basal media +PSB), T4 (Basal media + VAM), T5 (Basal media + VAM + Azotobacter), T6 (Basal media + PSB + Azotobacter), T7 (Basal media + PSB + VAM), T8 (Basal media + PSB +VAM + Azotobacter)) and three replications at experimental farm of the department. Inoculation of growing media with biofertilizers individually or in combination significantly influenced the different growth parameters of seedlings in comparison to seedling raised in media without application of any biofertilizers. The application of biofertilizers in growing media proved beneficial for seedling growth. Combined application of PSB and Azotobacter resulted in maximum seedling vigour followed by application of Azotobacter alone, PSB+VAM and PSB + VAM + Azotobacter. However inoculation of growing media with Azotobacter alone, PSB + VAM and PSB + VAM + Azotobacter were also statistically equally good in terms of effect on seedling vigour.

Keywords: *Stevia*, vigour, performance etc.

Introduction
Stevia rebaudiana, also known as sweet leaf, or sugar leaf belongs to genus *Stevia* which consist of about 150 species of herbs and shrubs (Robert, 2010) [18], and is a member of the family Compositae and a native to Paraguay (Mark, 2009) [4]. Stevia is a non-caloric sweetener and that the sweet compounds pass through the digestive process of the body without chemically breaking down, hence making it a safe food substance for consumption by people who need to regulate their blood glucose level (Strauss, 1995) [22]. Stevia has been reported to have no adverse effect on humans (Brandle and Rosa, 1992) [3]. The leaves could be eaten fresh or when dried and it could be boiled in tea to release the sweetener. It has been used for centuries by the Guarani Indians of Paraguay, where the Plant originated from, as sweeteners for mate tea (Goettemoeller and Ching, 1999) [8]. Because of its commercial importance and poor seed fertility, the present study was carried out to study the effect of biofertilizers on seedling performance/Vigour of *Stevia rebaudiana.*

Material and Methods
The experiment was laid out in completely randomized block design with eight treatments (T1 (Basal media (Sand: Soil: FYM:: 1:1:1), T2 (Basal media + Azotobacter), T3 (Basal media +PSB), T4 (Basal media + VAM), T5 (Basal media + VAM + Azotobacter), T6 (Basal media + PSB + Azotobacter), T7 (Basal media + PSB + VAM), T8 (Basal media + PSB +VAM + Azotobacter)) and three replications at experimental farm of the department. The biofertilizers used in the experiment i.e. Azotobacter, Phosphorus Solubilising Bacteria (PSB) and Vascular Arbuscular Mycorrhizae (VAM) were procured from the Division of Microbiology, IARI, New Delhi. These biofertilizers were applied in growing media in different combinations during sowing of seeds as per the requirement of the treatments. Under each replication, only dark coloured seeds were sown.

Results and Discussion
Under this experiment, data recorded on seedling height (cm), number of leaves per seedling, root length (cm), collar diameter (mm), leaf length (cm), leaf breadth (cm), leaves weight-fresh and dry (g/seedling), shoot weight-fresh and dry (g/seedling), root weight-fresh and dry

(g/seedling), whole seedling weight - fresh and dry (g/seedling) was found statistically significant and are presented in tables 1 and 2.

Seedling height in different treatments ranged between 19.59 to 38.70 cm. The maximum seedling height (38.70 cm) was recorded in T6 treatment, which was statistically at par with T2 (36.98 cm), T7 (37.17 cm) and T8 (35.27 cm) treatments. The minimum seedling height (19.59 cm) was recorded in T1 treatment in which no biofertilizers were applied (Table 1).

The number of leaves per seedling in different treatments ranged between 13.07 to 18.33. The maximum number of leaves per seedling (18.33) were recorded in T7 treatment which was statistically at par with T2 (17.27), T3 (17.80) and T6 (18.03) treatment. The minimum number of leaves per seedling (13.07) were recorded in T1 treatment in which no biofertilizers were applied (Table 1).

Table 1: Effect of biofertilizers on the growth and development of seedlings

Treatments	Seedling height (cm)	Number of leaves/seedling	Root length (cm)	Collar diameter (mm)	Leaf length (cm)	Leaf breadth (cm)
T1 (Basal media (Sand: Soil: FYM:: 1:1:1))	19.59	13.07	6.82	1.78	4.10	2.05
T2 (Basal media + Azotobacter)	36.98	17.27	9.57	3.61	8.90	4.00
T3 (Basal media +PSB)	28.85	17.80	9.93	2.78	7.19	2.99
T4 (Basal media + VAM)	25.09	14.47	11.23	2.40	6.63	2.77
T5 (Basal media + VAM + Azotobacter)	31.63	15.1	10.40	2.77	7.43	3.26
T6 (Basal media + PSB + Azotobacter)	38.70	18.03	10.85	3.40	8.49	3.74
T7 (Basal media + PSB + VAM)	37.17	18.33	12.32	3.50	8.28	3.25
T8 (Basal media + PSB +VAM + Azotobacter)	35.27	16.10	11.68	3.34	7.66	3.31
CD 0.05	3.58	1.43	2.20	0.26	0.69	0.32
SE±	1.67	0.67	1.03	0.12	0.32	0.15

Root length ranged between 6.82 to 12.32 cm among treatments. The root length was found maximum (12.32 cm) in T7 treatment which was statistically at par with T4 (11.23 cm), T5 (10.40 cm), T6 (10.85 cm) and T8 (11.68 cm) treatment and minimum root length (6.82 cm) was recorded in T1 treatment (Table 1).

The collar diameter of seedlings in different treatments ranged between 1.78 to 3.61 mm. The maximum collar diameter of seedling (3.61mm) was observed in T2 treatment which was statistically at par with T6 (3.40 mm) and T7 (3.50 mm) treatments and minimum collar diameter (1.78 mm) was recorded in T1 treatment in which no biofertilizers were applied (Table 1).

Leaf length was found between 4.10 cm to 8.90 cm in different treatments. The maximum leaf length (8.90 cm) was recorded in T2 treatment which was however statistically at par with T6 (8.49 cm) and T7 (8.28 cm) treatment and minimum leaf length (4.10 cm) was recorded in T1 (basal media) treatment (Table 1).

Fig 1: Biofertilizer effect on seedlings of *Stevia rebaudiana*

T1 (Basalmedia (Sand: Soil: FYM; 1:1:1))
T3 (Basalmedia +PSB)
T5 (Basalmedia +VAM+Azotobacter)
T7 (Basalmedia + PSB +VAM)
T2 (Basalmedia +Azotobacter)
T4 (Basalmedia +VAM)
T6 (Basalmedia + PSB +Azotobacter)
T8 (Basalmedia + PSB +VAM+Azotobacter)

The leaf breadth in different treatments ranged between 2.05 to 4.00 cm. The maximum leaf breadth (4.00 cm) was recorded in T2 treatment which was statistically at par with T6 (3.74 cm) treatment and minimum leaf breadth (2.05 cm) was recorded in T1 treatment in which no biofertilizers were applied (Table 1).

The fresh leaves weight per seedling ranged between 1.03 to 4.58 g. The maximum fresh leaves biomass (4.58 g) per seedling was recorded in T6 treatment and minimum (1.03 g) in T1 treatment. The fresh shoot weight per seedling was recorded between 0.44 g to 3.10 g in different treatments with the maximum value (3.10 g) in T6 treatment and minimum (0.44 g) in T1 treatment.

Fresh root biomass in different treatments ranged from 0.53 to 1.83 g/seedling. The fresh root weight per seedling was maximum (1.83 g) in T2 treatment which was statistically at par with T6 (1.75 g), T7 (1.77 g) and T8 (1.76 g) treatment and minimum fresh root weight per seedling was recorded in T1 (0.53 g) treatment. The whole seedling fresh weight per seedling in different treatments was between 2.00 to 9.45 g. The maximum seedling fresh weight (9.45 g) was recorded in T6 treatment which was statistically at par with T2 (8.38 g) and T7 (8.43 g) treatment. The minimum whole seedling fresh weight (2.00 g) was recorded in T1 treatment (Table 2).

The dry leaves weight per seedling in different treatments ranged between 0.17 to 0.70 g. The maximum dry leaves weight (0.70 g /seedling) was recorded in T6 treatment which was statistically at par with T2 (0.66 g/seedling) treatment and minimum dry leaves weight (0.17 g/seedling) was recorded in T1 treatment (Table 2).

The dry shoot weight per seedling ranged between 0.08 to 0.52 g in different treatments. The maximum dry shoot weight (0.52 g/seedling) was recorded in T6 treatment which was statistically at par with T2 (0.51 g/seedling) and T7 (0.48 g/seedling) treatment. Minimum dry shoot weight per seedling (0.08 g) was recorded in T1 treatment (Table 2).

Table 2: Effect of biofertilizers on the seedling vigour at nursery stage

Treatments	Leaves weight(g)/ seedling		shoot weight (g)/seedling		root weight (g)/ seedling		Whole seedling weight (g)	
T1 (Basal media (Sand: Soil: FYM:: 1:1:1))	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
T2 (Basal media + Azotobacter)	1.03	0.17	0.44	0.08	0.53	0.08	2.00	0.33
T3 (Basal media +PSB)	3.70	0.66	2.86	0.51	1.83	0.31	8.38	1.48
T4 (Basal media + VAM)	2.69	0.40	1.38	0.27	1.20	0.20	5.26	0.86
T5 (Basal media + VAM + Azotobacter)	1.86	0.29	1.00	0.16	0.88	0.17	3.75	0.62
T6 (Basal media + PSB + Azotobacter)	2.79	0.45	1.90	0.32	1.25	0.24	5.94	1.01
T7 (Basal media + PSB + VAM)	4.58	0.70	3.10	0.52	1.75	0.32	9.45	1.54
T8 (Basal media + PSB +VAM + Azotobacter)	3.86	0.56	2.81	0.48	1.77	0.34	8.43	1.37
CD 0.05	0.56	0.11	0.47	0.06	0.56	0.09	1.28	0.23
SE±	0.26	0.05	0.22	0.03	0.26	0.05	.0.60	0.11

The dry root weight per seedling in different treatments was recorded between 0.08 to 0.34 g. The maximum dry root weight per seedling (0.34 g) was recorded in T7 and T8 treatment which was statistically at par with T6 (0.32 g), and T2 (0.31 g) treatments and minimum dry root weight per seedling (0.08 g) was recorded in T1 treatment (Table 2).
The whole seedling dry weight ranged between 0.33 to 1.54 g per seedling in different treatments. The maximum whole seedling dry weight per seedling (1.54 g) was recorded in T6 treatment which was statistically at par with T2 (1.48g), T7 (1.37g) and T8 (1.31 g) treatments. Minimum dry seedling weight per seedling (0.33g) was recorded in T1 treatment in which no biofertilizers were applied (Table 2).
The soil is a habitat for a vast, complex and interactive community of soil organisms, whose activities largely determines the chemical and physical properties of the soil and growth of the plant. From seed germination until a plant reaches maturity, it lives in close association with soil organisms. This association is termed as rhizocoenosis (Lynch, 1983) [12]. The vast majority of plant associated soil organisms inhabit the rhizosphere, defined as the zone around roots in which bacterial growth is stimulated by the release of nutrients. Within the rhizosphere, there is a continuous interaction between plant roots and the rhizosphere organisms that comprise the rhizosphere. These interactions can have an important influence on plant growth. They may be viewed as associative (or neutral), harmful or beneficial (Saxena and Tilak, 1994) [19].
Critical analysis of the results of present study revealed that inoculation of growing media with biofertilizers (*viz*. Azotobacter, PSB and VAM) individually or in combination significantly influenced the different growth parameters of seedlings in comparison to seedling raised in media without application of any biofertilizers. However the significant effect of different parameters was different for different combination of biofertilizers. Among individual inoculation of biofertilizers, Azotobacter resulted in higher seedling height (36.98 cm), collar diameter (3.61 cm), leaf length (8.90 cm), leaf breadth (4.00 cm), fresh leaf weight (3.70 g/ seedling), dry leaf weight (0.66 g/seedling), fresh shoot weight (2.86 g/seedling) and dry shoot weight (0.51 g/seedling), fresh root weight (1.83 g/seedling) and dry root weight (0.31 g/seedling) and whole seedling fresh weight (8.38 g/seedling) and whole seedling dry weight (1.48 g/ seedling). The beneficial effect of single inoculation of Azotobacter is well documented in literature (Gupta *et al*, 2010, Saxena and Tilak, 1994, Paroha *et al*, 2009) [9, 19, 16]. In a study under Bangalore condition in *Stevia rebaudiana* Das *et al* (2007) [6] found similar results. Among individual biofertilizers they also found higher biomass production in Azotobacter treatment.
In the present study, combined inoculation of biofertilizers (*viz*. Azotobacter, VAM and PSB) in different combinations significantly influenced the plant performance/vigour of *Stevia rebaudiana* resulted in improved growth and development (Table 1 and 2) in comparison to control. The seedling raised in media containing PSB and Azotobacter (T6 treatment) resulted in higher values for seedling height (38.70 cm), fresh leaves weight (4.58 g/seedling), dry leaves weight (0.70 g/seedling), fresh shoot weight (3.10 g/seedling), dry shoot weight (0.52 g/seedling), fresh whole seedling weight (9.45 g) and dry whole seedling weight (1.54 g) in comparison to plants raised in media without application of biofertilizers (basal media). Similar findings have been reported by Paroha *et al.* (2009) [16] for the maximum seedling height of teak contained in media containing PSB and Azotobacter. Likewise in *Allium sativum*, Chattoo *et al.* (2007) [4] concluded that inoculation of PSB and Azotobacter significantly increased the plant height and weight per plant.
Also in *Viola pilosa*, Thakur (2003) [23] reported that combined application of FYM+ PSB+ Azotobacter gave maximum plant height, fresh herb yield and dry herb yield. Ocampo *et al.* (1975) [15] studied interaction between Azotobacter and PSB in *Lavander spica* and observed that plant growth was greatest when seedling were inoculated with both micro organisms. The present findings may be attributed to the fact that Azotobacter which is well known for its capacity to increase growth by fixing atmospheric nitrogen and also produce growth promoting substances like auxins and gibberellins (Mohammad and Prasad, 1998 in *Eucalyptus camaldulensis*). Similarily PSB solubilizes the soil phosphates and results in increased growth and yield in the combination.
In the present investigation, application of Azotobacter alone in basal media recorded maximum collar diameter (3.61mm), maximum leaf length (8.90 cm) and maximum leaf breadth (4.00 cm) and maximum fresh root weight (1.83 g per seedling). The higher growth and yield parameters may be due to proliferation of inoculated and other beneficial micro organism which might have affected the plant growth by nitrogen fixation, mobilization of soil phosphates by providing growth promoting metabolites that stimulate plant and by suppression of pathogenic micro organism as reported by Kennedy and Chillapillai (1998) [11].
The application of PSB+ VAM in basal media resulted in maximum number of leaves (18.33), maximum root length (12.32 cm) and dry root weight (0.34 gm/ seedling). This synergistic host response could be mainly due to the fact that PSB can release some phosphate ions from otherwise

sparingly soluble phosphates sources (Barea *et al*. 1983) [2] and it was postulated that VAM fungal hyphae can tap these ions and translocate them to plants (Azcon *et al*. 1986) [1]. Also PSB survived longer around micorrhizal than non micorrhizal roots and some times acted synergistically with the micorrhizal fungus to increase plant growth (Singh, 1990) [21]. *Centrosema pubescens* also reported well to dual inoculation of VAM and PSB in rock phosphate amended soils (Saxena and Tilak, 1994) [19]. Arbascular micorrhiza symbiosis is known to promote acquisition of mineral nutrients especially phosphorus by host plants. Enhancement in growth of VAM treated plants may possibily be due to mineralization of organic phosphorus by VAM fungi. PSB also mineralizes insoluble phosphorus present in soil and makes available to plants for their growth and development (Verma *et al*, 2008) [24].

The combined application of PSB + VAM + Azotobacter also recorded maximum dry root weight (0.34 g/seedling). Similar findings have been reported by Das *et al.* (2007) [6] in which combined application of Azotobacter, VAM and PSB resulted in 48% increase in biomass yield in *Stevia rebaudiana* and by Earanna (2007) [7]. This might be due to the combined application of biofertilizers which caused maximum fixation of nitrogen, increased uptake of soil P and K by stevia plants (Das *et al*, 2008) [5]. Nitrogen and phosphorus are the two major plant nutrients and the combined inoculation of nitrogen fixers and phosphate solubilising micro organism may benefit plants better than either group alone. In the present study also combined inoculation of nitrogen fixer (Azotobacter) and PSM (PSB) resulted in higher values for almost all the characters which is evident from the maximum seedling biomass (fresh and dry) among all the treatments. The beneficial effect of combined inoculation of Azotobacter and PSB is also well documented in literature (Saxena and Tilak, 1994) [19].

If our goal is to maximize beneficial plant growth responses, then optimal combination of selected microbes should be used. It is important, therefore, to identify the best strains of beneficial microbes, verify their compatibility and combined efficacy, both *in vitro* and *in vivo*, and employ this combination inoculums in real agricultural situations as part of the management and production practices. Although the results of field tests have demonstrated the considerable potential of using combined inoculums of microorganisms to promote plant growth but the consistency of results is lacking. It can be because the underlying mechanisms accounting of the phenomenon are not well understood. In case of growth benefit by dual inoculation, it is assumed that each beneficial microbe contributes something towards enhanced plant growth, such as increased nitrogen or soluble phosphate.

The mechanisms may be much more complex than that and elucidating them should be subject of future research. Furthermore, some of the bacteria involved may be interacting on more than one metabolic level i.e. Phosphorus solubilizers may also be auxin producers, and N2 fixers may also solubilize phosphorus. It is well known that certain soil properties such as moisture holding capacity, pH, texture and organic matter content favour the establishment, survival and activity of certain organisms.

Eric Randy and R. Politud (2016) [17] concluded that propagating *Stevias* using shoot tip cuttings with 1/3 garden soil + 1/3 sand + 1/3 vermicast media mixture using plastic cups for two months showed the best growth performance. Singh and Verma in 2015 [20] finally concluded that growing media significantly influenced the survival rate of cutting, growth and development parameter of stevia sapling in which media vermicompost + soil + FYM was best media since the survival of cutting and development parameters were higher than those on the other media, therefore this result suggested that vermicompost + soil + FYM should be used as a growing media. Ma Claudia *et al.* concluded that S*tevia* can be propagated vegetatively using cuttings treated with IBA 7.4 mM or ANA 6.4 mM + IBA 0.3 mM, preferable in the period from February to July, with the exception of June.

Conclusion

- Inoculation of growing media with biofertilizers individually or in combination significantly influenced the different growth parameters of seedlings in comparison to seedling raised in media without application of any biofertilizers.
- Among individual effect of each biofertilizers (Azotobacter, PSB and VAM), inoculation with Azotobacter alone in the growing media resulted in higher seedling vigour as compared to the individual effect of VAM, PSB and control.
- Among individual as well as combined inoculation of different biofertilizers, Azotobacter + PSB inoculation resulted in maximum seedling vigour. However inoculation of growing media with Azotobacter alone, PSB + VAM and PSB + VAM + Azotobacter were also statistically equally good in terms of effect on seedling vigour.

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