Elevation of plant growth parameters in two solanaceous crops with the application of endophytic fungus

S.B. Sarbadhikary and N.C. Mandal*

Mycology and Plant Pathology Laboratory, Department of Botany, Visva-Bharati, Santiniketan-731 235, West Bengal, India.
Received: 05-09-2017 Accepted: 18-05-2018

ABSTRACT

In the present study VBEF2, an endophytic fungal strain of Aspergillus isolated from the leaf of Schima wallichii (DC.) Korth. showed excellent plant growth promoting (PGP) attributed both in-vitro and in-vivo conditions. It was found to possess good phosphate solubilizing and IAA producing ability as it produced soluble phosphate and IAA upto 418.33 µg ml⁻¹ and 19.8 µg ml⁻¹, respectively in the seven days kinetic studies. It also exhibited siderophore producing attribute with remarkable antifungal activities against potential plant pathogens Fusarium oxysporum, Colletotrichum acutatum and Penicillium digitatum. It showed excellent survivability in alluvial soil in laboratory condition at a temperature range of 28°C to 50°C. The strain when subjected to field application enhanced various growth and yield parameters significantly (p < 0.05) in Solanum lycopersicum (tomato) and Solanum melongena (brinjal).

Key words: Antifungal activity, Endophyte, Field application, IAA production, Phosphate solubilization.

INTRODUCTION

Use of bio fertilizers is a healthy practice in crop cultivation as it completely avoids the chance of environmental pollution caused by the chemical fertilizers. Chemical pesticides and fertilizers can cause huge damages in an ecosystem through the process of bioaccumulation and biomagnifications due to their synthetic nature. Therefore improvement of crop production using biofertilizers has become an eco-friendly tool in the agriculture sector through the modern era researches. Bio fertilizers can improve the crop health by nitrogen fixing (Islam et al., 2013), phosphate solubilizing ability (Bajpai and Sundara, 1971; Ghosh et al., 2016) or some time they promote plant growth by producing plant growth promoting factors like auxin (IAA) and gibberellins (Reetha et al., 2014; Kang et al., 2014). Beside these direct plant growth promoting (PGP) qualities, bio fertilizers some time shield the crop plants through their antagonistic activities against a wide range of plant pathogens (Morales et al., 2008; Nourozian et al., 2006). Plant growth promoting rhizobacteria (PGPR) (Vessey, 2003), vesicular arbuscular mycorrhiza (VAM) (Abbasi et al., 2015) and blue green algae (BGA) (Singh et al., 2014) are mostly used in this regard. Some researchers also reported the use of endophytic organisms in agricultural fields for crop improvement (Ngamau et al., 2014). In the present endeavour the PGP characters of a previously isolated endophytic strain of Aspergillus and its plant growth promotion in Solanum lycopersicum (tomato) and Solanum melongena (brinjal), were checked.

MATERIALS AND METHODS

Selection of endophyte: Among the several endophytic fungal strains isolated from the leaves of Schima wallichii (DC.) Korth., strain VBEF2 was found to be good antagonism against two wilt causing bacteria under in-vitro and in-vivo conditions and identified as a strain of Aspergillus through microscopic characterizations (observations yet to be published). Therefore the present study deals with the in-vitro PGP characterizations and field applications of VBEF2 for the plant growth promotions.

Plant growth promoting activities of VBEF2: The plant growth promoting abilities like phosphate solubilization, IAA production and siderophore production of the strain were studied following standard protocols.

Siderophore production of VBEF2 was checked using Chrome Azurol S (CAS) agar medium. The endophyte was streaked in a malt extract agar medium (MEAM) plate amended with CAS and incubated at 28°C for 72 h (Schwyn and Neilands, 1987).

Qualitative phosphate solubilization ability of VBEF2 was checked using Pikovskaya’s (PVK) medium, pH 7.0 (Pikovskaya, 1948) containing tricalcium phosphate as a source of insoluble phosphate. Chen reagent (Chen et al., 1956) was used for analyzing the kinetics of phosphate solubilization for 7 days. Simultaneously pH of the PVK broth inoculated with VBEF2 was checked for 7 days. Test fungus was not inoculated in the negative control set.

*Corresponding author’s e-mail: mandalnc@rediffmail.com
Indole 3-acetic acid (IAA) producing ability of VBEF2 was checked by colorimetric method using Salkowski reagent (Tsavkelova et al., 2007) both in the presence and absence of L-tryptophan (500 mg ml⁻¹). The kinetic of the IAA production by the strain VBEF2 was studied for the consecutive seven days and IAA production was recorded.

**Antifungal activity of VBEF2:** The isolate VBEF2 was thoroughly grown in the malt extract broth (MEB) and centrifuged at 8000 RPM for 10 min for obtaining the Cell free supernatant (CFS). The antifungal potential of the CFS was checked against *Fusarium oxysporum* MTCC2480, *Penicillium digitatum* VBCS1 and *Colletotrichum acutatum* MTCC2074 by agar well diffusion method (Fernandez-Garayzabal et al., 1992). The fungal strains were either laboratory isolate or procured from Microbial Type Culture Collection (MTCC), IMTech, Chandigarh. Fluconazole (200 µg ml⁻¹) and sterilized water were used as positive and negative control, respectively. Three replications were used for each treatment.

**Survivability of endophyte in the soil samples:** Survivability of VBEF2 was checked for a span of one year in alluvial soil samples. Two ml of the culture having spore suspension of 5.8×10⁵ conidia ml⁻¹ was mixed with 10 g of autoclaved alluvial soil collected from tomato field. Soil samples containing fungal strain were incubated at three different temperatures viz., 28ºC, 37ºC and 50ºC for one year duration. The survivability of the strain was checked after every three months during the experiment.

**Field application of the endophyte:** The *in-vivo* application of endophyte was carried out in tomato F1 Hybrid Lakshmi and brinjal variety VNR-218 in Binuria village (23°39.951’N, 87°37.971’E) of Birbhum, West Bengal, India. Average annual rainfall and mean temperature of the experimental site was 1480 mm and 36.9ºC (during April) to 11.7ºC (during January), respectively. The texture of the soil was alluvial type. Two sets viz., VBEF2 treated and un-inoculated control were designed and each set having sixteen and nine plants for tomato and brinjal, respectively. Sterilized un-inoculated ME broth was sprayed in control plants, whereas two litre of fully grown culture of VBEF2 (4.8×10⁴ conidia ml⁻¹) in ME broth was sprayed on the treatment plants. The endophytic fungus was applied thrice in the rhizosphere region of the plants. First treatment was given at the seedling stage and the next two were given after 15 days and 30 days of the first spray. Data on phytomorphometric and yield were recorded and compared after the sufficient growth of the plants.

**Statistical analysis:** Paired t-test was carried out at 95% Confidence Interval of the Difference (p < 0.05) for the analysis of the outcomes of the field study using Statistical Package for the Social Sciences (SPSS) software. Microsoft (MS) Excel program version, 2007 was used for the data interpretation of the other experiments (at least three replicates).

**RESULTS AND DISCUSSION**

**Plant growth promoting activities of VBEF2:** In the siderophore production assay VBEF2 confirmed its siderophore production ability by developing a light orange halo zone surrounding its colony (Fig. 1a). Siderophores are iron chelating compounds produced by the microorganisms for uptaking of ferric (Fe³⁺) ions from the soil environment. Siderophore producing organisms can exclude other microorganisms from its local soil environment which may compete for iron (Haas and Défago, 2005). Thus, siderophore production and suppression of phytopathogens of a strain has significant agricultural importance. Till now many siderophore producing microorganisms have been reported (Maksimov et al., 2011; Hussein et al., 2014).

Phosphate solubilization of VBEF2 was noticed by the production of clear hollow zone around the fungal growth in the PVK plate (Fig.1b). The kinetic study elucidated a positive correlation between the soluble phosphate production by the strain and the pH reduction of the fungal culture during the experiment. The highest phosphate solubilization of VBEF2 was found at the 7th day as the soluble phosphate production reaches 418.33±3.21 µg ml⁻¹.
with the pH of 3.17±0.04 (Fig. 2a). Till now very few reports are published regarding the phosphate solubilizing ability of endophytic species of *Aspergillus*. Previously, ten endophytic strains of *Aspergillus* which were collected from the Microbial culture collection, Regional Plant Resource Center, Bhubaneswar, Odisha showed phosphate solubilizing activities (Sahoo and Gupta, 2014). However, their sources have not been mentioned in the report. In this context, the ability of phosphate solubilization by the endophytic *Aspergillus* strain VBEF2 of *Schima wallichii* is significant. The strain was also found to produce IAA strictly in the presence of L-tryptophan and the production gradually enhanced with the increasing time. Maximum amount of IAA production was observed on the 7th day of the experiment as the value reached up to 19.8±0.53 µg ml⁻¹ (Fig. 2b). It was also noticed that the strain could not produce IAA in the absence of L-tryptophan.

**Antifungal potential of VBEF2:** The CFS of VBEF2 produced prominent zones of inhibition against all the plant pathogenic fungi used and confirmed its antifungal efficiency (Fig. 3). The endophyte showed highest activity against *Penicillium digitatum* by producing 19.33±0.57 mm zone of inhibition. The diameter of zones of inhibition produced by the CFS against *Fusarium oxysporum* and *Colletotrichum acutatum* were found to be 16±1 mm and 10.33±0.57 mm, respectively. All the three fungi were sensitive to Fluconazole as clear zones appeared in the positive control treatment and resistant to sterilized water. Antifungal efficiency of VBEF2 throws light on its applicability as biocontrol agent in the agriculture sector.

**Survivability of the endophyte in soil samples:** It was found that the endophyte survived quite well throughout the one year observation period (Table 1). At 28ºC the isolate was found to survive till the end while at 37ºC its survivability was not noticed during the 12th month of the experiment. Its presence in the soil sample at 50ºC was observed till the 6th month of the assay but 9th month onwards it lost its survivability. These findings clearly indicate that the endophytic fungal strain have enough potential to survive in the soil samples in a wide range of temperature. Since the endophyte was isolated from the leaf tissue, checking its survivability in the soil samples was necessary. So its ability to be viable in the soil at three different temperatures opened the gateway for its application in the natural soil conditions.

**Field application of the endophyte:** During recording the field data, VBEF2 was found to elevate the growth as well as the yield in both tomato and brinjal plants significantly (p < 0.05) (Fig. 4). The results recorded were more promising in brinjal as in VBEF2 treatment the plant height and number of branches plant⁻¹ were found to be 92.88±4.64 cm and 53.77±4.23 as compared to control i.e. 69.11±2.14 cm and 36.33±3.27, respectively. On the other hand, the endophyte application enhanced them by 5.81% and 22.48% in tomato. Other growth parameters viz., number of fruit plant⁻¹, average weight of fruit and total weight of fruit plant⁻¹ were also found more in the treatments of both the crops (Table 2). In tomato the yield was found to be 3045.32 g per m² and 1678.95 g per m² in VBEF2 treated and control sets, respectively. The yield obtained from the brinjal plants was also in the same fashion as the values were 6914.35 g per m² and 2814.32 g per m² in treatment and control set.

---

**Table 1:** Survivability of VBEF2 (conidia ml⁻¹) in alluvial soil samples at different temperature.

| Temperature | Incubation period | 0 month | 3 month | 6 month | 9 month | 12 month |
|-------------|-------------------|---------|---------|---------|---------|---------|
| 28ºC        |                   | 5.8x10⁵ | 5.6x10⁵ | 3.5x10⁵ | 8.1x10⁴ | 9.2x10³ |
| 37ºC        |                   | 5.8x10⁵ | 5.1x10⁴ | 3.8x10³ | 2.7x10³ | 0       |
| 50ºC        |                   | 5.8x10⁴ | 3.1x10³ | 2.8x10³ | 0       | 0       |

---

Fig 3: Antifungal activity of VBEF2.

![Antifungal activity of VBEF2](image)

Fig 4: Field application of VBEF2. (a)Treatment set in tomato. (b) Control set in tomato. (c) Treatment set in brinjal. (d) Control set in brinjal.
Table 2: Response of VBEF2 treatments on different growth parameters of brinjal and tomato.

| Growth parameters                        | VBEF2 treatment | Control | VBEF2 treatment | Control |
|------------------------------------------|-----------------|---------|-----------------|---------|
| Average height of plants (cm) ±SD        | 92.88±4.64      | 69.11±2.14 | 85.37±1.75      | 80.68±7.90 |
| Number of branches plant\(^{1}\) ±SD     | 53.77±4.23      | 36.33±3.27 | 45.93±2.67      | 37.5±3.77 |
| Number of fruit plant\(^{1}\) ±SD        | 37.22±3.15      | 19.44±2.50 | 63.87±4.28      | 46.56±4.60 |
| Average weight of fruit (g) ±SD          | 185.77±10.34    | 144.77±8.65 | 47.68±1.66      | 36.06±3.94 |
| Total weight of fruit plant\(^{1}\) (kg) ±SD | 6.40±0.29      | 2.55±0.27  | 3.12±0.18       | 1.45±0.24  |
| Total yield (g per m\(^{2}\))             | 6914.35         | 2814.32  | 3045.32         | 1678.95   |

Table 3: Pair t-test analysis among the treatment and control sets in tomato and brinjal.

| Growth parameters | Plant assessed | Mean | Std. Deviation | Std. Error | Paired Differences | 95% Confidence Interval of the Difference | t | df | Sig. (2-tailed) |
|-------------------|----------------|------|----------------|------------|-------------------|----------------------------------------|---|----|----------------|
|                   |                | Lower | Upper         |            |                   |                                        |   |     |                |
| Average height of plant (cm) | Tomato | 4.68750 | 8.29232 | 2.07308 | .26884 | 9.10616 | 2.261 | 15 | .039 |
| Number of branches plant\(^{1}\) | Brinjal | 23.7778 | 5.11805 | 1.70602 | 19.84370 | 27.71186 | 13.938 | 8 | .000 |
| Number of fruit plant\(^{1}\) | Tomato | 8.43750 | 4.47167 | 1.11792 | 6.05472 | 10.82028 | 7.548 | 15 | .000 |
| Average weight of fruit (g) | Brinjal | 17.7778 | 4.43784 | 1.47928 | 14.36555 | 21.18901 | 12.018 | 8 | .000 |
| Total weight of fruits plant\(^{1}\) (kg) | Tomato | 11.62500 | 4.63141 | 1.15785 | 9.15709 | 14.09291 | 10.040 | 15 | .000 |
| Total yield (g per m\(^{2}\)) | Brinjal | 41.00000 | 12.04159 | 4.01386 | 31.74401 | 50.25599 | 10.215 | 8 | .000 |

Paired t-test analysis of the field results in tomato and brinjal using SPSS respectively. Paired t-test analysis strongly supports the outcomes of the field experiment (Table 3).

In summary, the endophyte exhibited excellent PGP attributes like phosphate solubilization, IAA production and siderophore production. It also showed excellent antifungal activity against *Penicillium digitatum*, *Fusarium oxysporum* and *Colletotrichum acutatum* to a great extent. After confirming the prolonged survivability in the soil samples in three different temperatures sets viz., 28ºC, 37ºC and 50ºC for one year duration the endophyte was applied in the field condition. During the *in-vivo* field application the strain was found to elevate plant growth as well as yield parameters in tomato and brinjal.

**CONCLUSION**

In this present endeavour the isolate VBEF2 showed excellent PGP activities like phosphate solubilization, IAA production and siderophore production. Previously, few reports were published regarding the plant growth promoting ability of some endophytic species of *Aspergillus*. For instance, an endophytic strain of *Aspergillus terreus* was found to promote the plant growth in *Helianthus annuus* and lessen the adverse effect of the stem rot (Waqas et al., 2015). As the use of chemical fertilizers in the crop field is deeply hazardous (Britto and Girija, 2006), thus enhancement of plant growth and qualities using biofertilizers has become an effective and eco-friendly tool nowadays. In the present study the endophytic isolate VBEF2 has significantly promoted the plant growth and yield parameters in two solanaceous crops in field condition. Secondly, eradication of plant pathogens and confer protection to the crop plants are also the indirect ways to promote plant growth. The endophyte VBEF2 was found to show excellent antifungal efficiency against the plant pathogenic fungi used which indicates its ability to save the crops from the pathogenic stresses. Due to having these plant growth promoting features the endophytic fungal isolate VBEF2 can thus be used as a biofertilizer for better cultivation.

**ACKNOWLEDGEMENT**

Authors are thankful to University Grants Commission (UGC) for the financial support.

**REFERENCES**

Abbasi, Hisamuddin, Akhtar, A. and Sharf, R. (2015). Vesicular Arbuscular Mycorrhizal (VAM) Fungi: A Tool for Sustainable Agriculture. *American Journal of Plant Nutrition and Fertilization Technology*, 5: 40-49.

Bajpai, P.D. and Sundara, R.W.V.B. (1971). Phosphate solubilizing bacteria III. Soil inoculation with phosphate solubilizing bacteria. *Soil Science and Plant Nutrition*, 17: 46–53.

Britto, A. J. De. and Girija, L. S. (2006). Investigations on the effect of organic and inorganic farming methods on black gram and green gram. *Indian Journal of Agricultural Research*, 40: 204 - 207.
Chen, Jr.P.S., Tonbara, T.Y. and Warner, H. (1956). Microdetermination of phosphorus. *Analytical Chemistry*, 28: 1756-1758.

Fernandez-Garayzabal, J.F., Delgado, C., Blanco, M., Vazquez-Boland, J.A., Briones, V., et al. (1992). Role of potassium tellurite and brain heart infusion in expression of the hemolytic phenotype of *Listeria* spp. on agar plates. *Applied and Environmental Microbiology*, 58: 434-438.

Ghosh, R., Barman, S., Mukherjee, R. and Mandal, N.C. (2016). Role of phosphate solubilizing *Burkholderia* spp. for successful colonization and growth promotion of *Lycopodium cernuum* L. (Lycopodiaceae) in lateritic belt of Birbhum district of West Bengal, India. *Microbiological Research*, 183: 80-91.

Haas, D. and Défago, G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Reviews Microbiology*, 3: 307–319.

Hussein, K. A. and Joo, J. H. (2014). Potential of siderophore production by bacteria isolated from heavy metal: polluted and rhizosphere soils. *Current Microbiology*, 68: 717-723.

Islam, M.R., Sultana, T., Joe, M.M., Yim, W., Cho, J.C. and Sa, T. (2013). Nitrogen-fixing bacteria with multiple plant growth-promoting activities enhance growth of tomato and red pepper. *Journal of Basic Microbiology*, 53: 1004–1015.

Kang, S.M., Khan, A.L., You, Y.H., Kim, J.G., Kamran, M. and Lee, I.J. (2014). Gibberellin production by newly isolated strain *leifsonia soli* se134 and its potential to promote Plant Growth. *Journal of Microbiology and Biotechnology*, 24: 106–112.

Maksimov, I.V., Abizgil’dina, R.R. and Pusenkova, L.I. (2011). Plant growth promoting rhizobacteria as alternative to chemical crop protectors from pathogens (Review). *Applied Biochemistry and Microbiology*, 47: 333–345.

Morales, H., Sanchis, V., Usall, J., Ramos, A.J. and Marin, S. (2008). Effect of biocontrol agents *Candida sake* and *Pantoea agglomerans* on *Penicillium expansum* growth and patulin accumulation in apples. *International Journal of Food Microbiology*, 122: 61–67.

Ngamau, C.N., Matiru, V.N., Tani, A. and Muthuri, C.W. (2014). Potential use of Endophytic Bacteria as Biofertilizer for Sustainable Banana (*Musa* spp.) Production. *African Journal of Horticultural Science*, 8:1-11.

Nourozian, J., Etebarian, H.R. and Khodakaramian, G. (2006). Biological control of *Fusarium graminearum* on wheat by antagonistic bacteria. *Journal of Science Education and Technology*, 28: 29–38.

Pikovskaya, R.I. (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Microbiology*, 17: 362-370.

Reetha, S., Bhuvaneswari, G., Thamizhiniyan, P. and Mycin, T.R. (2014). Isolation of indole acetic acid (IAA) producing rhizobacteria of *Pseudomonas fluorescens* and *Bacillus subtilis* and enhance growth of onion (*Allium cepa* L.). *International Journal of Current Microbiology and Applied Sciences*, 3: 568-574.

Sahoo, H. R. and Gupta, N. (2014). Evaluation of phosphate solubilising potential of some endophytic fungi under solid and liquid State. *BMR Microbiology*, 1: 1-6.

Schwyn, B. and Neilands, J.B. (1987). Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry*, 160: 47-56.

Singh, S., Singh, B.K., Yadav, S.M. and Gupta, A.K. (2014). Potential of Biofertilizers in Crop Production in Indian agriculture. *American Journal of Plant Nutrition and Fertilization Technology*, 4: 33-40.

Tsavkelova, E.A., Cherdynseva, T.A., Botina, S.G. and Netrusov, A.I. (2007). Bacteria associated with orchid roots and microbial production of auxin. *Microbiological Research*, 162: 69–76.

Vessev, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*, 255: 571-586.

Waqas, M., Khan, A. L., Hamayun, H., Shahzad, R., Kang, S. M., Kim, J.G. and Lee, I. J. (2015). Endophytic fungi promote plant growth and mitigate the adverse effects of stem rot: an example of *Penicillium citrinum* and *Aspergillus terreus*. *Journal of Plant Interactions*, 10: 280-287.