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ORIGINAL ARTICLE

Phyllospheric Bacterial Treatments Improve Growth in *Helianthus annuus* L.

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ABSTRACT

The current study focuses on the effect of variation in time of bacterial treatment during different growth phases of seeds on plant growth improvement using *Helianthus annuus* L. For this purpose, two phyllospheric auxin-producing bacterial strains *Bacillus thuringiensis* (E4) and *Lysinobacillus fusiformis* (E6) were used for bacterial treatment. Two experiments were conducted in the present work with pre-germination and post-germination inoculation treatments to seeds. A comparative analysis was made in order to find out the efficiency of *Bacillus thuringiensis* (E4) and *Lysinobacillus fusiformis* (E6) for improving growth of *Helianthus annuus* L. Post-germination treatments were found effective with *Bacillus thuringiensis* (E4) but *Lysinobacillus fusiformis* (E6) worked well in both treatments i.e., in pre-germination and in post-germination inoculation treatment.

Keywords:
Pre-germination, post-germination, Bacillus, Lysinobacillus, Helianthus annuus L.

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INTRODUCTION

Bacterial communities associated with plants are diverse in nature and colonize below or above ground plant surfaces[1]. The three most significant factors in plant-microbe interactions include the plant compatibility, soil profile, survival ability of microbes[2]. Phyllosphere refers to the aerial parts of the plants that are colonized by the microbes and is affected by various biotic and abiotic factors which can alter within hours, days or with seasons, thus the phyllosphere is the habitat with extremes of environment. Among the population of microbes that reside in this region, bacteria are the most dominant microbial group[3]. Phyllosphere is significant and valued bacterial habitat. The phyllospheric bacteria are variable in number mostly ranging from 10^6-10^7 cells per cm^2 on the surface of leaf[4]. Bacteria that dwell aerial parts of plants i.e., phyllospheric bacteria, improve the ecosystem and biogeography of plants by improving plant performance under the influence of extreme environmental conditions. Plant growth promoting phyllospheric bacteria show an ancient and prevalent symbiotic relation that influence various growth and developmental processes of plants in various ways like hormones production i.e., indole-3-acetic acid, secreting secondary metabolites, synthesizing siderophores and mineral solubilization, release of other growth promoting substances and protection against various infectious diseases of plants that promote plant productivity[5,6]. Use of plant growth promoting bacteria for growth promotion is an effective and efficient method in comparison with herbicides, pesticides, fungicides, and insecticides in agriculture that impose many hazardous effects on plants and human health[7]. For integrated solutions of agriculture, microbial inocula are potential constituents that are also used for various environmental issues. Plant health is maintained by inoculants that help in plant growth promotion, nutrient availability, and uptake hence improves plant growth. Phyllospheric bacteria are suitable to be used as biofertilizers since these can tolerate severe environmental conditions so can be effectively utilized in plant growth promotional studies[8]. The present study aims at the comparative analysis of plant growth improvement in *Helianthus annuus* L. following pre-germination and post-germination inoculation treatments with phyllospheric isolates i.e., *Bacillus thuringiensis* (E4) and *Lysinobacillus fusiformis* (E6).

MATERIALS AND METHODS

Bacterial Growth Requirements

Two phyllospheric bacterial strains i.e., *Bacillus thuringiensis* (E4) and *Lysinobacillus fusiformis* (E6) that were already isolated and identified by Mir [9] and Batool [10] were used in the current study. L-agar medium was used to routinely culture the isolated bacterial strains at 37ºC for 24 hours.

Bacterial Treatment Experiment

Certified seeds of *H. annuus* L. (var. RAH3) were obtained from Punjab Seed Corporation, Lahore. Seeds were surface sterilized by following Fatima and Ahmed [11]. Pre-germination and post-germination treatments were given to the sterilized seeds.

Pre-Germination Inoculation Treatment

Pre-germination inoculation treatments were given to the seeds before germination by following Ahmed and Hasnain[12] and were transferred in the respectively labeled pots. Pots were kept in light (16 hours duration and 10K lux) at 25 ± 2^{o}Cand watering was done regularly. Plants were harvested after 20-25 days of growth and growth parameters of inoculated and non-inoculated plants were observed.

Post-Germination Inoculation Treatment

Seeds were inoculated with bacterial strains after seed germination in the same way as in pre-germination inoculation treatment following Ahmed and Hasnain[12] and were transferred in the respectively labeled pots. Pots were kept in light (16 hours duration and 10K lux) at 25±2^{o}C and watering was done regularly. Plants were harvested after 20-25 days of growth and growth parameters of inoculated and non-inoculated plants were observed.

Biochemical Analysis

For biochemical analysis of treated and non-treated plants (pre-germination and post- germination inoculation treatments), Lowry *et al.*[13] methods were used for soluble protein estimation and pigment contents were measured following the method of Vaishnav *et al.*[14].

RESULTS

Pre-germination Inoculation Treatment

Pre-germination inoculation treatment with the bacterial isolates *Bacillus thuringiensis* (E4) and *Lysinobacillus fusiformis* (E6) stimulated growth of *H. annuus* L. Almost 11.9 and 14.1% increment in shoot length and 55.7 and 79 % increase in length of roots of the inoculated plants was observed with *Bacillus thuringiensis* (E4) and *Lysinobacillus fusiformis* (E6) respectively, when compared with non-inoculated plants. Both isolates improve the number of leaves and plant growth as compared to control plants i.e. 4.6%. Both bacterial strains enhanced fresh weight upto 8.06% with *Bacillus thuringiensis* (E4) and 1.41% with *Lysinobacillus fusiformis* (E6) treatment as compared to control plants (Fig. **2**). Chlorophyll content of the plants was improved by pre-germination inoculation treatment. *Lysinobacillus fusiformis* (E6) caused improvement in both chlorophyll "a" and chlorophyll "b" i.e., 8.12 and 85.1% respectively, as compared to the control plants. Total chlorophyll was improved up to 47.4% with *Lysinobacillus fusiformis* (E6) inoculation whereas *Bacillus thuringiensis* (E4) has no effect on chlorophyll content of the plants. Increase in soluble protein content was recorded up to 129 and 89% with *Bacillus thuringiensis* (E4) and *Lysinobacillus fusiformis* (E6) respectively as compared to non-inoculated plants (Fig. **3**).

Post-Germination Inoculation Treatment

In post-germination inoculation treatment, bacterial inoculation with *Bacillus thuringiensis* (E4) and *Lysinobacillus fusiformis* (E6) has shown 7.45 and 13.9% increment in shoot length respectively, over control. Similarly, enhancement in root length was observed with *Bacillus thuringiensis* (E4) and *Lysinobacillus fusiformis* (E6) up to 38.6 and 60.6% in comparison to control plants

(Fig. **1**). Both strains showed increases in the leaf number when compared with the control up to 4.6% (Fig. **1**). The increment in fresh weight up to 8.12% was recorded with *Lysinobacillus fusiformis* (E6), whereas *Bacillus thuringiensis* (E4) has no effect on the fresh weight of the plants (Fig. **2**). Biochemical analysis was also carried out and it was observed that chlorophyll 'a' content of inoculated plants was improved up to 146.2 and 101% with *Bacillus thuringiensis* (E4) and *Lysinobacillus fusiformis* (E6) respectively, in comparison with control. Similarly, enhancement in chlorophyll 'b' was recorded as compared to control, i.e., 73.5 and 73% respectively. Total chlorophyll content was improved i.e., 95.6 and 78.2% with *Bacillus thuringiensis* (E4) and *Lysinobacillus fusiformis* (E6) respectively, in comparison to control (Fig. **3**). Soluble protein content was also recorded after inoculation with both strains. Both strains showed pronounced improvement in protein content up to 153 and 170.7% respectively, over control (Fig. **4**).

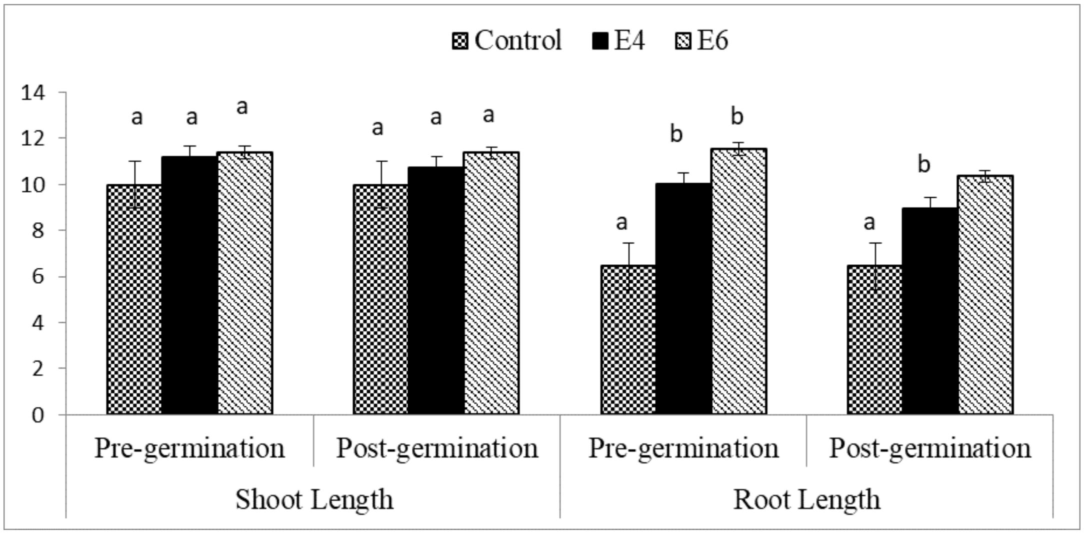

Figure 1: Effect of pre-germination and post-germination bacterial treatments on shoot length (cm) and root length (cm) of *H. annuus* L. [E4-*Bacillus thuringiensis;* E6- *Lysinobacillus fusiformis*].

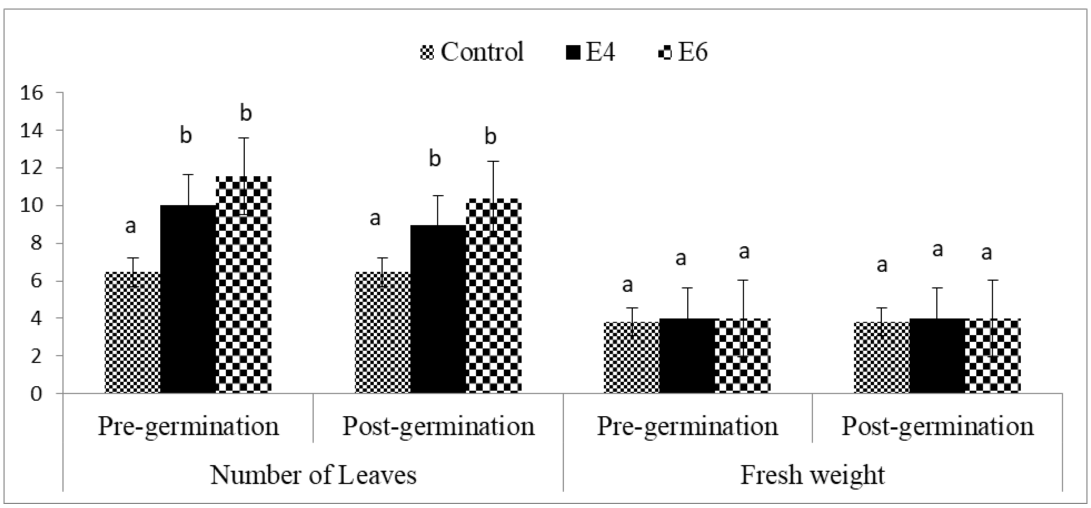

Figure 2: Effect of pre-germination and post-germination bacterial treatments on a number of leaves and fresh weight (g) of *H. annuus* L. [E4-*Bacillus thuringiensis;* E6- *Lysinobacillus fusiformis*].

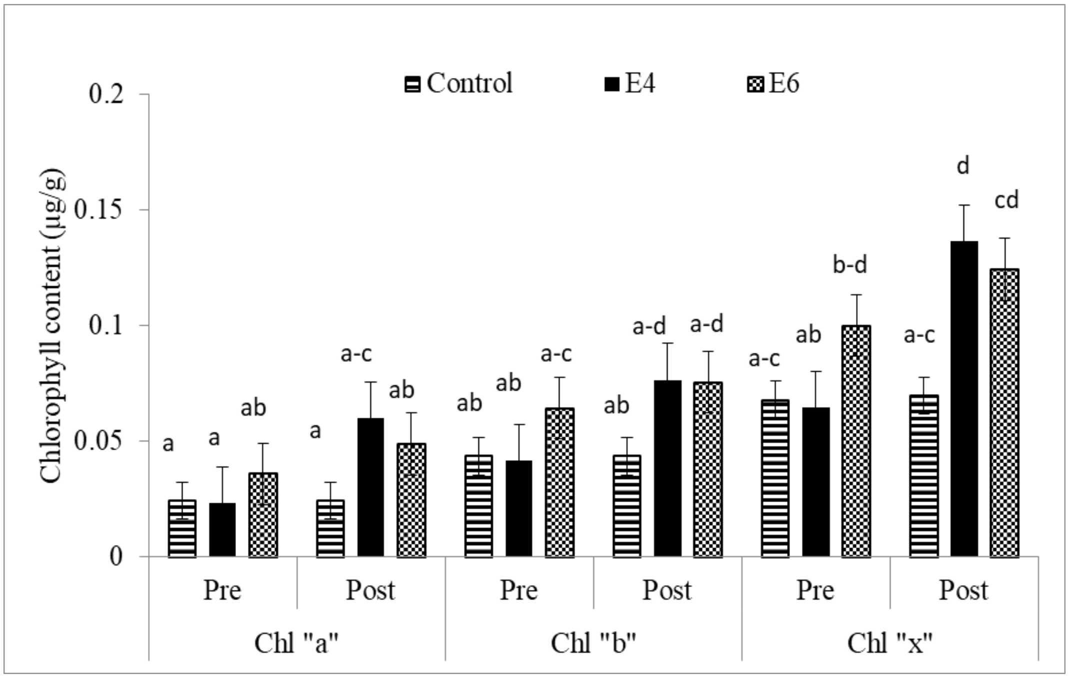

Figure 3: Effect of pre-germination and post-germination bacterial treatments on chlorophyll content of *H. annuus* L. [Pre-Pre-germination, Post-Post-germination, Chl "a"- chlorophyll 'a'; Chl "b"- chlorophyll 'b'; Chl "x"- total chlorophyll content; E4-*Bacillus thuringiensis*; E6- *Lysinobacillus fusiformis*].

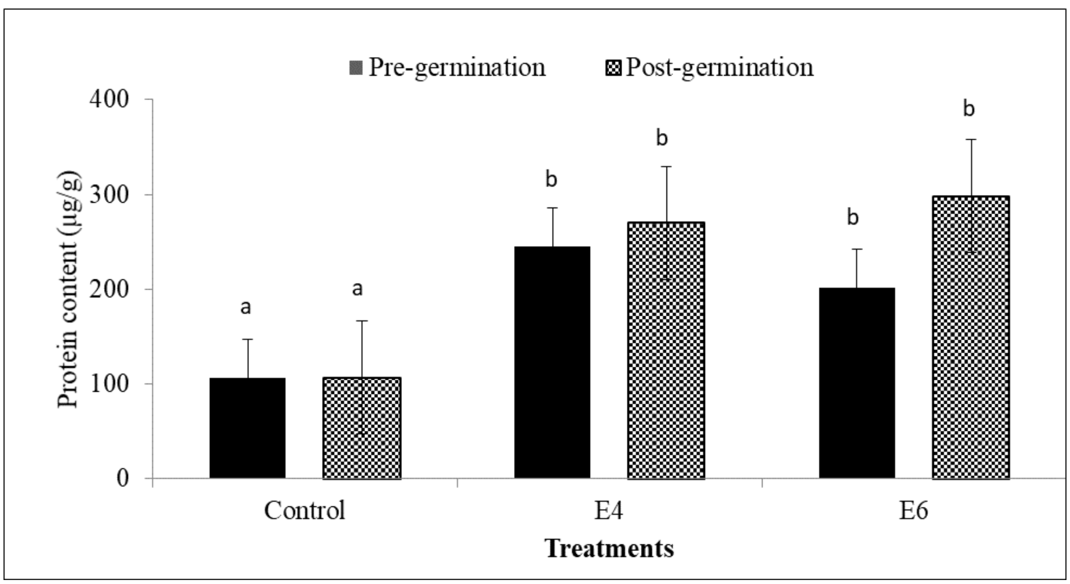

Figure 4: Effect of pre-germination and post-germination bacterial treatments on the protein content of *H. annuus* L. [E4- Bacillus thuringiensis, E6- Lysinobacillus fusiformis].

DISCUSSION

Two auxin-producing phyllospheric bacterial strains *Bacillus thuringiensis* (E4) and *Lysinobacillus fusiformis* (E6) were utilized to determine the influence of bacterial treatment on growth of a plant by using pre-germination and post-germination inoculation treatments. In pre-germination inoculation treatment, *Bacillus thuringiensis* (E4) significantly enhanced almost all growth parameters like root length, shoot length, number of leaves and fresh weight up to 11.8, 55.7, 8.06 and 4.6% respectively, while in post-germination bacterial inoculation, *Bacillus thuringiensis* (E4) significantly enhanced the growth of plants by increasing growth parameters like shoot length and root length up to 7.5, 38.6 and 4.6% as compared to the control (Fig. **1**). *Bacillus thuringiensis* (E4) improved plant growth in post-germination inoculation treatment only but *Lysinobacillus fusiformis* (E6) proved to be efficient in both pre-germination and post- germination inoculation treatments. Auxins control various growth and developmental processes in plants by direct or indirect mechanisms of actions. The positive impact of auxin-producing bacteria on plant growth has been reported through various studies[12]. Noumavo and his colleagues also reported that shoot length improvement may be due to the auxin activity that plays important role in plants shoot elongation. Cytokinins also play a key role in improving plant growth and shoot length through cell division and differentiation that is responsible for cell elongation, hence improve shoot length [15]. Bacterial auxin is responsible for the production of the better secondary root system to improve root surface area and is responsible for improving nutrient absorption capacity of the plants [6]. Plant growth promoting bacteria improve plant growth by improving plant growth parameters like shoot length, root length, number of leaves and fresh weight as compared to control plants[16]. In pre-germination inoculation treatment, *Bacillus thuringiensis* (E4) did not show any positive effect on chlorophyll content whereas in post-germination treatment, chlorophyll 'a', chlorophyll 'b' and total chlorophyll content were significantly increased up to 146.2, 75.5 and 95.7% respectively, by E4 treatment. Significant improvement in protein content with *Bacillus thuringiensis* (E4) after pre-germination and post germination inoculation treatments was recorded up to 129.7 and 153% respectively. Inoculation with *Lysinobacillus fusiformis* (E6) showed a significant increase in chlorophyll and protein content in both pre- and post-germination inoculation treatments. *Lysinobacillus fusiformis* (E6) improve total chlorophyll, chlorophyll "a" and chlorophyll "b" contents in pre-germination inoculation treatment, as well as enhancement in protein content, was observed i.e., 47.5, 85.1, 47.4 and 89% respectively in comparison with control. Similarly, in post-germination inoculation treatment, significant enhancement up to 100, 73, 78.2 and 170% were recorded respectively in chlorophyll "a", chlorophyll "b", total chlorophyll content and protein content with *Lysinobacillus fusiformis* (E6) treatment (Fig. **3** & **4**). The results revealed that plant growth promoting phyllospheric bacteria significantly enhanced plant growth by increasing chlorophyll as well as protein content. High chlorophyll content resulted in efficient photosynthesis thus ultimately results in growth promotion of plants. In *Sullacarnosa*, better photosynthesis was recorded by improving overall chlorophyll content due to bacterial inoculation[17]. In the runner bean, improvement in total protein contents of inoculated plants was observed as compared to control plants[18].

CONCLUSION

The results of these experiments using phyllospheric auxin- producing bacteria i.e., *Bacillus thuringiensis* (E4) and *Lysinobacillus fusiformis* (E6) are comparable with the plant growth improvement observed using auxin-producing rhizobacterial treatment. This shows that auxin-producing phyllospheric bacteria have similar phytostimulatory potential as exhibited by auxin-producing rhizobacteria[19]. The comparative analysis showed that the growth phase of seed at the time of bacterial inoculation affects plant growth improvement. *Bacillus thuringiensis* (E4) was more effective in improving *H. annuus* growth when seeds were treated with bacterial inoculum at seedling stage but *Lysinobacillus fusiformis* (E6) treatment was equally effective both when used for treatment before or after seed germination.

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