

# Effect of growth media and plant growth promoting rhizobacteria (PGPR) on growth and flowering indices of China aster

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**Effect of growth media and plant growth promoting rhizobacteria (PGPR) on growth and flowering indices of China aster**

**Abstract:** The China aster is used for various purposes like bouquets preparation, flower arrangements, bedding plants, edge, and herbaceous borders in gardens, flower shows and exhibitions. The study focused on using different growth media and plant growth-promoting rhizobacteria (PGPR) to enhance the growth of China aster. Four types of growth media: garden loamy soil (S), soil + perlite (SP), soil + cocopeat (SC), and soil + cocopeat + perlite (SCP), with three incubation conditions: inoculation with *Bacillus subtilis* (Ehrenberg 1835) Cohn 1872 and *Pseudomonas putida* Trevisan 1889, and a control group with no bacterial incubation, each with three replicates. Regardless of the growth media used, *P. putida* resulted in taller plants than those treated with *B. subtilis*. Among the growth media tested, SCP produced the tallest plants and most axillary shoots. SCP with *P. putida* had the highest chlorophyll content and leaf area. SCP also resulted in the most flowers, especially with *P. putida*. SCP with *P. putida* had the highest leaf nitrogen content, while SC and SP with *B. subtilis* showed high leaf phosphorus and potassium levels. The findings from our study highlight that utilizing SCP as a composite growth media, with or without bacterial incubation, produced the most favorable effects on the growth and flowering indices of China aster.

**Key words:** *Bacillus subtilis*, coco peat, perlite, *Pseudomonas putida*, soil

**Učinek gojišča in rast vzpodbujajočih rizobakterij (PGPR) na rast in cvetne indekse kitajske nebine**

**Izvleček:** Kitajska nebina se uporablja v različne namene kot so izdelava šopkov, cvetnih aranžmajev, kot pokrovna cvet-ica in za obrobe v vrtovih, za cvetlične predstave in razstave. Raziskava se je osredotočila na uporabo različnih rastnih medijev in rast vzpodbujajočih rizobakterij (PGPR) na pospeševanje rasti kitajske nebine. Uporabljeni so bili štiri rastni mediji in sicer: vrtna zemlja (S), vrtna zemlja + perlit (SP), vrtna zemlja + ostanki kokosa (SC), in vrtna zemlja + ostanki kokosa + perlit (SCP), s tremi režimi inokulacije: inokulaciji z bakterijama *Bacillus subtilis* (Ehrenberg (1835) Cohn 1872 in *Pseudomonas putida* Trevisan 1889 in kontrola brez bakterijske inokulacije, vse v treh ponovitvah. Ne glede na rastne medije je inokulacija z bakterijo *P. putida* dala višje rastline kot tista z bakterijo *B. subtilis*. Med preiskušanimi rastnimi mediji so bile rastline na SCP najvišje, z največ stranskih poganjkov. Rastline na SCP z bakterijo *P. putida* so imele največjo vsebnost klorofila in največjo listno površino, imele so največ cvetov in največjo vsebnost dušika v listih. Rastline na gojiščih SC in SP, inokulirane z bakterijo *B. subtilis*, so imele v listih veliko vsebnost fosforja in kalija. Izsledki raziskave pojasnjujejo, da ima uporaba SCP kot sestavljenega gojišča, z ali brez inokulacije z bakterijami, najugodnejši vpliv na rast in cvetne indekse kitajske nebine.

**Ključne besede:** *Bacillus subtilis*, ostanki kokosa, perlit, *Pseudomonas putida*, tla

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## 1 INTRODUCTION

The China aster serves multiple functions, including for creating bouquets, flower arrangements, as bedding plants, and for edging herbaceous borders in gardens, as well as being a popular choice for flower shows and exhibitions. Plant growth-promoting rhizobacteria (PGPR) were proposed by Kloepper *et al.* (1980) for the first time, when he used fluorescent *Pseudomonas* as a growth promoter capable of resisting plant pathogens. Since then, the term PGPR has included all rhizobacteria effective in enhancing plants growth. PGPR plays a special role by slowing down plant contagion with disease, increasing nutrient absorption, and improving seed germination and plant resistance to environmental stresses. Rhizobacteria improve plant growth and synthesize some secondary metabolites such as phytohormone, enzymes, siderophores, and antibiotics (Ahmadi *et al.* 2020).

*Bacillus* and *Pseudomonas* are bacteria genera that frequently colonize the surface of the roots and the adjacent soil in the root zone (Ahemad and Kibret, 2014). These bacteria have two effects on plant growth. They produce stimulating plant growth compounds and help to absorb essential elements such as nitrogen by biological nitrogen fixation and phosphorous by solving phosphate. They also produce hormones including auxin, cytokinin, and gibberellin and the other effect is helping plants adaptable to biotic and abiotic stresses by inducing resistance systems (Sandhya *et al.* 2009; Orfanoudakis *et al.* 2010; Arzansh *et al.* 2012; Asif *et al.* 2019). Fernandez *et al.* (2020) stated that the use of growth-stimulating bacteria improved shoot and root length, stem diameter, dry mass, and absorption of nutritional elements. There are various beneficial effects of PGPRs on growth parameters in different plants. These effects include increasing leaf length, width, and leaf area, as well as enhancing flowering aspects like flower length, diameter, and numbers in chrysanthemum (*Chrysanthemum morifolium* L.) (Cipriano and Freitas, 2018). PGPRs also contribute to enhancing the fresh and dry mass of plants and flowers, flower stalk characteristics, stalk length, and the number of days to flowering in hyacinth (*Hyacinthus orientalis* L.) (Kumari *et al.*, 2016). Moreover, they can magnify bulb length, diameter, and weight in certain plants such as hyacinths (Karagöz *et al.*, 2019), and intensify plant height, vegetative and root mass, as well as leaf numbers in buttercup (*Ranunculus asiaticus* L.) (Domenico, 2020).

Growing media are an essential part of the propagation system because rooting competency depends on the type of medium used. The growing media

should be porous, uniform in texture, hold sufficient moisture, and should be well drained, which provides physical support, aeration, and water. Different types of growth media such as rock-wool, perlite, vermiculite, peat, and coconut fiber (coco peat) have been used to grow many kinds of crops (Bar-Tal *et al.* 2019). The use of different organic and inorganic growth media leads to the best nutrient uptake and sufficient growth, water, and oxygen holding. A good growth medium provides enough support to the plants, serves as a reservoir for nutrients and water, and improves gaseous exchange between the roots and atmosphere (Mazahreh *et al.* 2015). Perlite is a glassy volcanic rock with a rhyolitic composition and high water-holding capacity, but it has no buffering capacity and contains no mineral nutrients. It is a stable material, that can last for several years, and its stability is not greatly affected by acids and microorganisms (Bar-Tal, 2019). Cocopeat has been formed from the middle layers or mesocarp of the coconut fruits, which helps to absorb nutritional elements by plants (Carlile *et al.* 2019). Cocopeat has a high water-holding capacity and prevents from soil compaction, decreases germination time and increases seed germination uniformity (Yan and Murphy, 2008).

China aster (*Callistephus chinensis* Nees) belongs to the Asteraceae family comprising 152 species, which are widespread around the world. This plant is self-pollinated, propagated by seeds. The first flowering occurs within 55-60 days after planting. There is high diversity of flower colors in this ornamental plant including white, pale yellow, pink, red, blue, and violet. The most popular flower shape is a row of petals around the yellow center, but also with some rows of petals around the yellow center (Prasanth *et al.* 2020). This plant species is frequently used in green spaces as a bedding plant and in bouquets and floral compositions as a cut flower. Additionally, it is valued for its medicinal attributes, purportedly possessing anti-inflammatory, antioxidant, and anti-cancer qualities. (Bhargav *et al.* 2018). In order to the successful cultivation of some ornamental flowers such as marigold cultivars (Maślanka and Magdziarz, 2017), East liliun (Karagüzel, 2020) and gerbera (Sirin, 2011) growing media like perlite, peat moss, and cocopeat has been applied.

There is no literature for the application of PGPRs along with growth media on China aster as a bedding plant in landscaping and green spaces. Therefore, the objective of this study was to investigate the effects of two PGPRs of *B. subtilis* and *P. putida* and growth media of soil, soil + perlite, soil + cocopeat and their

combination on China aster growth, and flowering indices.

## 2 MATERIAL AND METHODS

### 2.1 STUDY AREA, PLANT CULTIVATION AND TREATMENT APPLICATION

The research was done at the Research Greenhouse of the Horticultural Science Department of the Agricultural Faculty of Zanjan University, Zanjan, Iran. The treatments included four types of growth media (soil (S), soil + perlite (SP), soil + cocopeat (SC) and soil + cocopeat + perlite (SCP)) and three types of incubation included incubation with plant growth promoting rhizobacteria, *B. subtilis* (Ehrenberg (1835) Cohn 1872 and *P. putida* Trevisan 1889, and no-bacterial incubation with three replicates. The seeds of Aadya cultivar were initially planted in plastic trays and later transplanted at the two-leaf stage into plastic pots. These pots were filled with various types of growing media, with one plant being placed in each pot.

At first, the plants in pots were irrigated fully. Then irrigation was once every second days. The incubation was with two types of bacteria using injection by siring with 108 colony-forming units per milliliter (CFU ml<sup>-1</sup>) into the growing media of the plants in the pots.

### 2.2 PLANT GROWTH AND FLOWERING INDICES

The height of plant, and flower diameter were measured by digital caliper. Leaf area was determined by leaf area meter. The number of axillary shoots and flower numbers were counted manually. The parameters of height and flowering were recorded once at the end of the experiment. The number of flowers were counted on the on the axillary stems.

### 2.3 TOTAL CHLOROPHYLL CONTENT

Total chlorophyll is defined as the sum of chlorophyll a and b. In this method 0.1 g of fully expanded leaf fresh sample was homogenized by 10 ml acetone 80%, and was centrifuged to obtain the extract. The absorbance of the extract was read by spectrophotometer at 645 and 663 nm wavelengths. Finally, the calculation of chlo-

rophyll a and b was using equations (1) and (2) (Arnon, 1949).

$$Chl\ a = [(12/7 \times A663 - 2/69 \times A645)] \times V/100 \times M \quad (1)$$

$$Chl\ b = [22/9 \times A645 - 4/69 \times A663] \times V/100 \times M \quad (2)$$

where Chl was chlorophyll (mg g<sup>-1</sup> FM), A663 and A645 relate to absorption rate at 663 and 645 nm wave lengths, respectively, V is the sample volume, and M is the fresh mass of the sample.

### 2.4 LEAF NITROGEN, POTASSIUM, AND PHOSPHOROUS

Nitrogen, potassium, and phosphorous of leaf samples were measured with Kjeldal device, flame photometer, and spectrophotometer, respectively (Tekaya et al. 2014).

### 2.5 STATISTICAL ANALYSIS

The experiment followed a 3× 4 factorial design within a completely randomized design with four replications. Data analysis was performed using SAS software version 9.4, and mean values were compared using the Duncan test at a significance level of 0.05. The graphs were plotted by Excel software.

## 3 RESULTS AND DISCUSSION

### 3.1 GROWTH AND FLOWER INDICES

Based on the interaction effects of growth media and type of bacteria incubation (Table 1), *P. putida* incubation increased plant height by 22.47% in soil + perlite (SP) growth media and had the biggest plant height (27.25 cm) as compared to the no-bacterial incubation treatment. Also, in soil + cocopeat + perlite (SCP) media, *P. putida* incubation and no-bacterial incubation treatment showed the same plant height and higher than *B. subtilis* incubation. *B. subtilis* incubation did not show an increasing effect on plant height in the treatments. Based on Table 1, generally, *P. putida* induced greater plant height than *B. subtilis*, and the application of SCP alone had a higher effect on plant height than other applied growth media. The application of three growth media together possibly provided better plant growth conditions and nutritional status than the other growth media treatments, which led to more longitudinal growth of the plants. An increase in plant height in media amended with different constituents has also been reported by

Singh (2013) in *Alstroemeria* and Rajera and Sharma (2017) in lily. A similar trend of increase in plant height was due to a nutrient rich media that was also recorded by Singh (2013) in tuberose plants. *P. putida* incubation has also been the most effective in plant height trait probably because pseudomonas species cause lower pH in soils than bacillus species (Ng et al. 2022), and China aster grows better in the soils with pH around 6.0 (*Indian Institute of Horticultural Research (ICAR)*). pH values will decrease due to the release of hydrogen ions because of an increase in soluble phosphate concentrations, however, the ability of pseudomonas species in phosphate solubility is higher than bacillus species (Ng et al. 2022).

SC media showed the smallest plant height (19.75 cm) regardless of the type of bacteria in comparison to the no-bacterial incubation treatment. Cocopeat as a growth media has a higher amount of water-holding capacity than perlite. Since China aster grows better in well-drained soils (*Indian Institute of Horticultural Research (ICAR)*), therefore, the possible reason for the decrease in most of the plant growth and flowering indices in SC growth media in this study might be the aeration status for the plants and the bacteria. The aeration and gas exchange between the soil and the atmosphere could be restricted for both the bacteria and the plants due to the application of SC with high water holding capacity and perhaps over-irrigating conditions. If the gas exchange rolls as a growth-reducing factor, growth parameters would be affected. Since China aster is a shallow-

rooted crop, it requires irrigation at an interval of 3 to 7 days depending on soil moisture (*Indian Institute of Horticultural Research (ICAR)*), it seems that watering every two days created over-irrigation conditions in SC growth media in our research. Lee et al. (2017) and Sharma and Godara (2017) reported that flowering time, fresh mass of plant, plant height, the number of flowers per bush, the size of flowers, and the earliest time of flowering was the highest in perlite + cocopeat media.

SCP media (with or without bacterial incubation) exhibited a greater number of axillary shoots compared to other media types (Table 1). Nevertheless, the combination of *P. putida* with S media resulted in the lowest number of axillary shoots, while SC media with *B. subtilis* and without bacterial incubation also displayed lower counts of axillary shoots. Due to the leaf production by axillary shoots, which require increased nutritional elements (Bredmose, 2003), the SCP media exhibited superior plant nutritional conditions compared to other media types, resulting in a higher number of axillary shoots.

SCP with or without bacterial incubation had the highest number and diameter of flowers among the treatments (Table 1), that is because SCP media had better plant nutritional condition than the other media (Bredmose, 2003) and led to more numbers and diameters of flowers. Also, this treatment had the highest number of axillary shoots as mentioned earlier, which could be considered rational because the more axillary shoots the more flowers. Axillary shoots serve as propagation mate-

**Table 1:** Effect of growth media and plant growth promoting bacteria on vegetative, flowering traits and chlorophyll content of China aster

Bacteria type	Culture media	PH (cm)	NAS	FN	FD (cm)	LA (cm <sup>2</sup> )	TChl (mg g <sup>-1</sup> F.M)
Without bacteria		22 .25bc	14 .50ef	13 .75de	5 .50bc	701 .80d	0 .754d
<i>Pseudomonas putida</i>	Soil	23 .25b	12 .00g	15 .00de	5.62bc	1063 .98b	0 .543efg
<i>Bacillus subtilis</i>		21 .50bc	15 .50e	15 .00de	4 .87d	577 .37f	1 .233ab
Without bacteria		23 .00b	14 .00ef	12 .75d	5 .62bc	890 .60c	0 .763d
<i>Pseudomonas putida</i>	Soil + perlite	27 .25a	16 .25cd	16 .50d	5 .75bc	639 .61e	0 .670e
<i>Bacillus subtilis</i>		23 .62ab	18 .75c	6 .25g	5 .37bcd	670 .28e	0 .616ef
Without bacteria		20 .25bc	12 .75g	11 .25ef	6 .00ab	698 .22de	0 .523g
<i>Pseudomonas putida</i>	Soil + cocopeat	19 .75c	16 .75cd	17 .25c	6 .00ab	534 .51f	0 .340h
<i>Bacillus subtilis</i>		19 .75c	12 .75g	12 .50e	4 .75d	528 .85f	0 .541efg
Without bacteria		27 .00a	23 .75b	25 .90a	6 .25ab	1121 .46ab	1 .112b
<i>Pseudomonas putida</i>	Soil + cocopeat + perlite	27 .00a	29 .50a	26 .00a	6 .37a	1495 .05a	1 .296a
<i>Bacillus subtilis</i>		23 .25b	23 .25b	23 .00b	6 .25ab	1143 .19ab	0 .863c

Mean value followed by the same letters in each column are not significantly different at 5 % level using Duncan multiple range test. PH: plant height, NAS: number of axillary shoots, FN: flower number, FD: flower diameter; LA: leaf area; TChl: total chlorophyll.

rial and give rise to flowering shoots, thereby enhancing flower production in terms of both quantity and diameter (Bredmose, 2003).

As shown in Table 1, SCP with *P. putida* had the greatest chlorophyll content ( $1.29 \text{ mg g}^{-1} \text{ FM}$ ) and leaf area ( $1495.05 \text{ mm}^2$ ). SC with both bacteria led to the lowest chlorophyll content and leaf area. That could be because of the aeration condition for plant growth in this media explained earlier. Reduction in chlorophyll content is directly related to environmental stresses such as low gas exchange between plant growth media and atmosphere, salinity etc. (Aslanpour et al. 2019). Consistently across the other treatments, a similar pattern was noted for chlorophyll content and leaf area as indicated in Table 1. This correlation aligns with the concept that greater leaf area tends to be associated with increased chlorophyll content (Aslanpour et al. 2019).

### 3.2 NITROGEN, PHOSPHOROUS, AND POTASSIUM

As demonstrated in Figure 1, SCP with *P. putida* had the greatest leaf nitrogen (N) ( $2.63 \text{ mg kg}^{-1} \text{ DM}$ ), SC plus *B. subtilis* induced to the greatest phosphorous (P) content ( $0.64 \text{ mg kg}^{-1} \text{ DM}$ ) and the highest potassium (K) content attributed to SP plus *B. subtilis* ( $2.44 \text{ mg kg}^{-1} \text{ DM}$ ). Normally, rhizobacteria help in fixing atmospheric nitrogen, and provide nutritional uptake by solubilizing phosphate and producing biologically active molecules (Arshad and Frankenberger, 1992). According to Figure 1, the trends in leaf nutrients for the treatments were uncertain and variable, making it difficult to draw definitive conclusions or provide explanations for these observations.

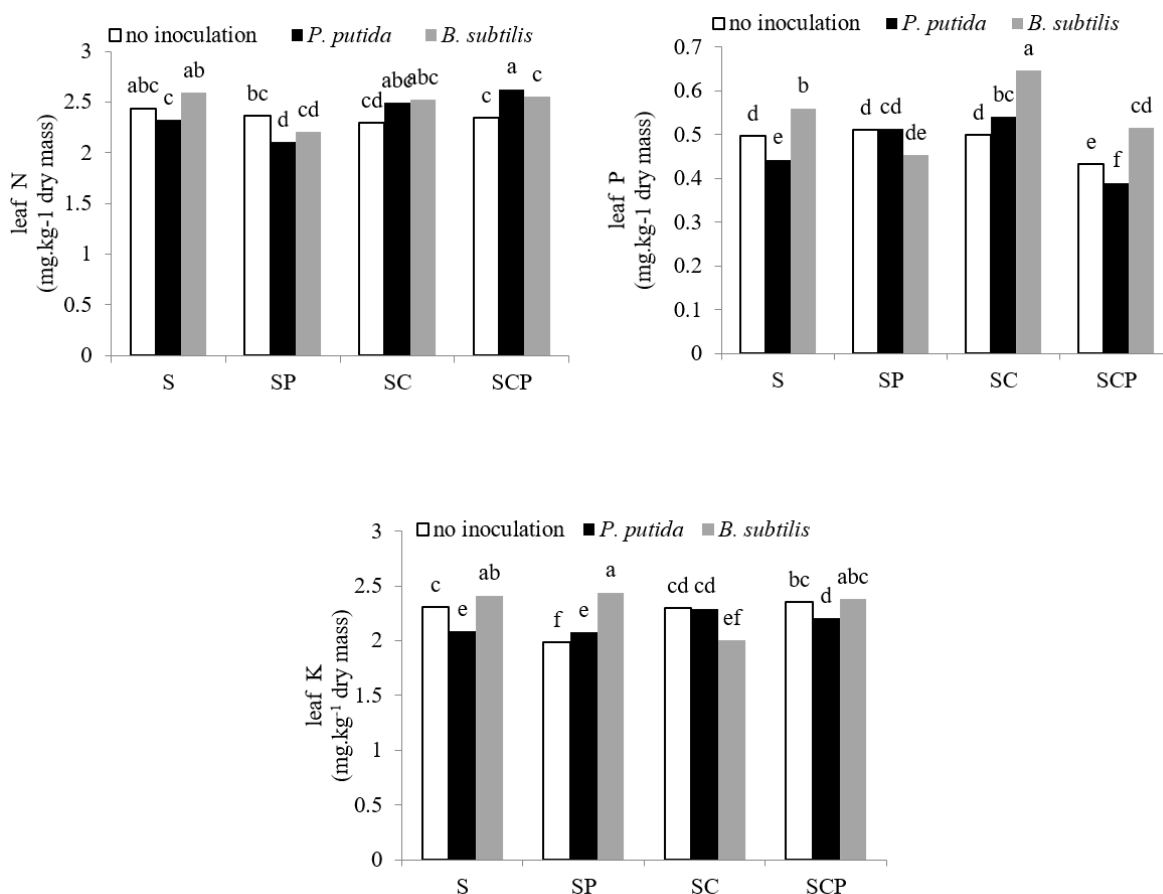


Figure 1: Effect of growth media and plant growth promoting bacteria on leaf nutrients (N, P, and K) of China aster. Mean value followed by the same letters in each column are not significantly different at 5% level using Duncan multiple range test. S: soil; SP: soil + perlite; SC: soil + cocopeat; SCP: soil + cocopeat + perlite



## 4 CONCLUSIONS

In summary, it can be inferred that SCP growth media, whether with or without bacterial incubation, had the most substantial influence on plant growth and flowering indices in China aster. The SCP media demonstrated superior performance in terms of plant height, number of axillary shoots, flower numbers and diameters, and leaf area.

## 5 DECLARATIONS

**Author contributions.** Conceived and designed the experiments: Arghavani, M and Aelaei, M. Performed the experiments: Mohammadi, S. Analyzed the data: Mohammadi, S and Sayyad-Amin, P. Wrote the paper: Sayyad-Amin, P, Edited the manuscript: Farahani, E and Esmaeili, S.

**Compliance with ethical standards (Conflict interest):** The authors declare that they have no conflict of interest.

**Data availability statement:** Data available on request from the corresponding author.

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