EFFECT OF INOCULATION WITH PGPR ON BASIL ANTIOXIDANT ACTIVITY

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Abstract
Basil (*Ocimum basilicum* L.) is a widely utilized culinary herb. It is used to flavour foods such as vegetables, meats, fish, etc. In traditional medicine is used for treatment of various disorders such as colds, respiratory diseases, cardiovascular diseases, metabolic and gastrointestinal disorders, etc. The effects of climate changes on agriculture can result in lower yield and nutritional quality of plants. The inoculation of plants with plant growth-promoting rhizobacteria (PGPR) such as *Azotobacter*, *Streptomyces*, and *Bacillus* are well known to lead to improvement in germination, growth, and yield. Also, it was found that PGPR enhance defence capacity of the plant. In this study, the basil seed were inoculated with selected PGPR isolates: Bac3, Azb, and Act. Control seeds were immersed into distilled water. After 6 weeks plant material was collected, and methanol extracts were prepared for antioxidant determinations. The changes in total phenol and flavonoid content, as well as antioxidant activity, were monitored. PGPR applied in the experiment have not cause significant changes in total phenol content. However, treatments with Azb and Act isolates have increased flavonoid content in basil plants. The antioxidant activity of basil plant has been measured as the ability of plant extracts to reduce DPPH radicals. Obtained results show that only treatment with Azb isolates significantly increases the antioxidant activity of basil plants. Results obtained in this study suggested that investigated isolates have different effects on the antioxidant characteristics of the basil plant. Further investigation is still needed to explore the possibility of using these PGPRs as potent bio-fertilizer in basil production.

Keywords: antioxidant activity, basil, inoculation.

Introduction
Basil (*Ocimum basilicum* L.) is a very popular medicinal plant but also is used as a culinary herb. Traditionally, it was used in the treatment of different diseases, like inflammation, bronchitis, tumours, etc. [1]. Results of scientific experiments support these traditional uses of the basil plant. Due to the high content of phenolic acids, flavonoids, and rosmarinic acid in leaf extract, but also aromatic compounds in essential oil basil have a wide spectrum of positive health properties [2]. In medicinal plants, the most investigated is an antioxidant activity because represents the first link between a chemical reaction and biological activity. It is generally accepted that free radicals are responsible for various damages in the body which precede different pathological conditions [3]. The antioxidant activity of phenolic compounds is primarily the result of their ability to be donors of hydrogen atoms and as such remove free radicals with the formation of less reactive phenoxyl radicals (Figure 1). The increased stability of the formed phenoxyl radical is attributed to electron delocalization and the existence of multiple resonance forms.

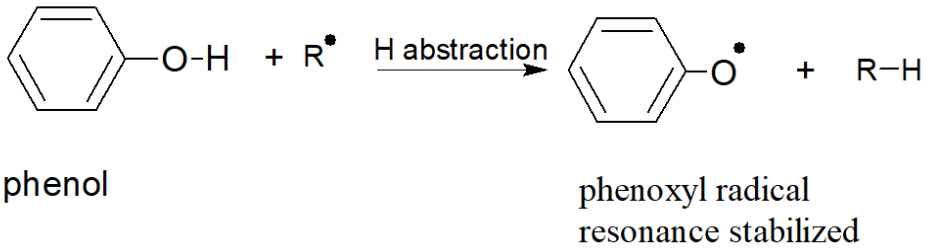

Figure 1. Radical reaction in which phenol as an antioxidant compound quenching an active free radical (**R**•) to **R - H** and itself converted into resonance stabilized phenoxyl radical.

In recent years, biochemical research on secondary biomolecules in plant extracts has received more and more attention due to the tendency to replace synthetic therapeutics with drugs of natural origin [4]. Also, several studies have shown that in response to stress conditions in plants are stimulated synthesis of phenolic compounds, which could enhance antioxidant activity of plant [5, 6].
The purpose of this study was to investigate the effect of PGPR on phenolic content and antioxidant activity of basil plants, in order to test potential of these PGPR isolates to induce accumulation of phenolic compounds in basil.

Experimental

Plant material and extract preparation

In this experiment, the bacterial strains were isolated from natural populations of nettle (*Urtica dioica* L.) rhizosphere soil. From selected PGPR organisms 3 different inoculums were prepared with isolates Bac3, Azb, and Act, and there were used for the inoculation of basil seeds. Plants were grown in controlled conditions for 6 weeks in pots with soil (Figure 2). The dried and powdered plant material was used for biochemical analyses. Methanol extracts were prepared by the method described in Šibul et al. [7] with minor modifications. About 1g of dried plant powder was soaked in 26 ml of methanol (80%) for 90 minutes at room temperature. The mixture was centrifugated at 3500 rpm/min for 10 minutes. The extraction was repeated 4 times with a fresh portion of solvent.

Figure 2. Pots with basil (*Ocimum basilicum* L.) plants at the end of experiment (ø control, and applied PGPR: isolate Azb, isolate Act, and isolate Bac3).

Total Phenolic Content

Total content of phenolics of a methanol extract was determined by the Folin-Ciocalteu procedure and expressed as mg of gallic acid equivalents per g dry weight plant material (mg GAE/g DW) [8]. A volume of 150 µL of distilled water and 50 µl of the corresponding extract were mixed with 1 ml of 0.1 M Folin-Ciocalteu reagent. After 10 minutes, in the mixture was added solution of 7.5% sodium carbonate and the absorbance was measured after 1h at 760 nm.

Total Flavonoid content

For determination of total flavonoid content (TFC) of methanol extracts method described by Chang et al. [9] was used. For analysis, 200 µL of the methanol extract was mixed with 800 µL distilled water, 100 µL of $AlCl_3$ 0.75M solution, and 100 µL 1M Na-acetate solution. After 30 minutes of incubation at room temperature in the dark, the absorbance was measured at 415 nm. The flavonoid content was calculated as a quercetin equivalent from the calibration curve of quercetin standard solutions and expressed as µg quercetin equivalent per g of dry weight plant material (µg QE/g DW).

Antioxidant activity

In order to assess the scavenging activity against DPPH· radical of methanol extracts, the method described by Sánchez-Moreno [10] was followed with minor modifications. The assay mixture contained 2 ml DPPH· solution (30 µmol/l) and 0.2 ml of the methanol extract in different concentrations (0.482 – 3.856 mg/ml). The mixture was shaken vigorously on a Vortex mixer, then incubated for 30 min at 25 °C in the dark, after which the absorbance of the remaining DPPH· was determined at 515 nm. For each sample, three replicates were carried out. Radical Scavenging Capacity (RSC) was calculated by the equation:

$RSC = (A_{control}-A_{sample})/A_{control} \times 100$

where $A_{control}$ is the control and A_{sample} is the sample solution absorbance. The concentration that causes a decrease in the initial absorbance (control) by 50% is defined as IC_{50}. The IC_{50} values for all RSC determinations were determined by polynomial fitting of the inhibition values using software ORIGIN 9.1.

Results and discussion

The TPC values of basil plant samples are given in Figure 3. Control sample had a TPC value of 4.24 mg GAE/g dw. The similar value was obtained for Azb treatment (4.18 mg GAE/g dw). Treatment with Act decreased the TPC value by about 25% (3.20 mg GAE/g dw). Similar TPC values for methanol extract of basil leaves have been reported [11].

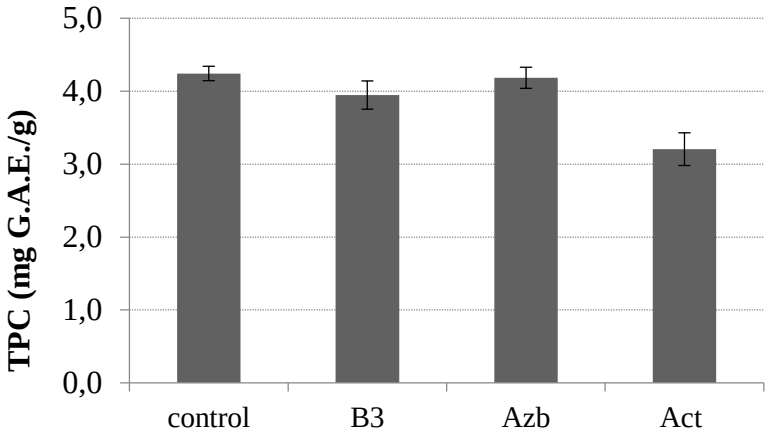

Fıgure 3. Effect of inoculation of PGPR on total phenolic content in basil plants

The TFC values were given in Figure 4. In control plants TFC value was 0.334 µg QE/g dw. The highest TFC value was found in plants inoculated with Azb (0.459 µg QE/g dw). Also, in plants inoculated with Act have been observed increase of TFC compared to control plants. According to the Ghorbanpour et al. [12] accumulation of flavonoids was stimulated by PGPRs inoculation.

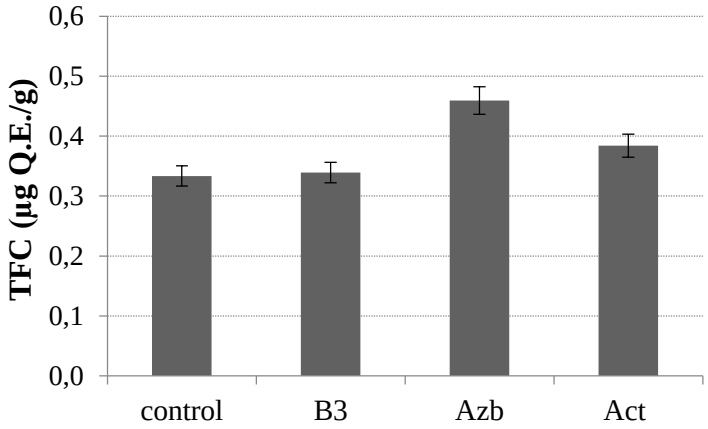

Figure 4. Effect of inoculation of PGPR on total flavonoid content in basil plants

The antioxidant activity of basil plant has been measured as the ability of plant extracts to reduce DPPH radicals (Figure 5) and was expressed as IC_{50} value. This value indicates concentration of extract which is required to achieve 50% scavenging activity. Obtained results show that only treatment with Azb isolates significantly increases the antioxidant activity of basil plants, with IC_{50} value 4.76 mg/ml compared to control (7.14 mg/ml).

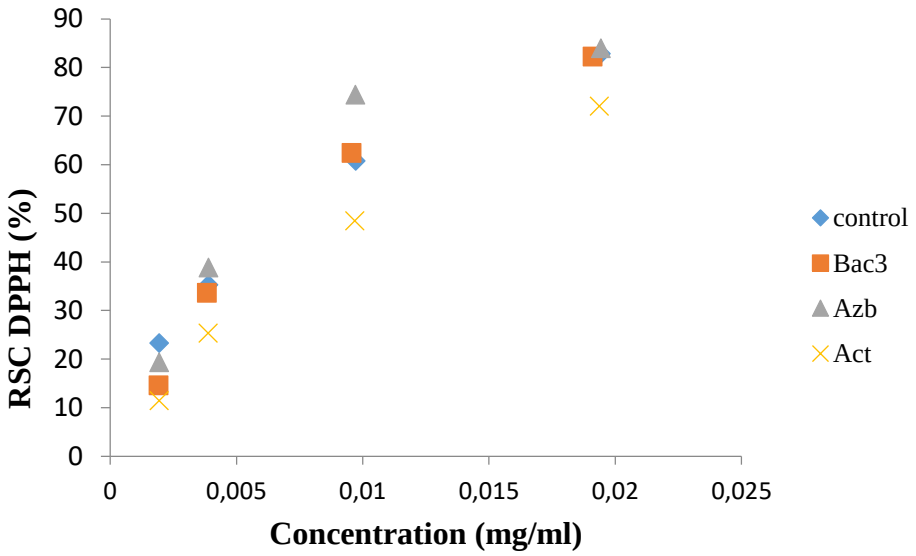

Figure 5. Effect of inoculation of PGPR on antioxidant activity of basil plants

Conclusion

Results obtained in this study suggested that investigated isolates have different effects on the antioxidant characteristics of the basil plant. Further investigation is still needed to explore the possibility of using these PGPRs as potent bio-fertilizer in basil production.

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