Pak. J. Bot., 45(S1): 529-534, January 2013.

EFFECT OF ACC DEAMINASE BACTERIA ON TOMATO PLANTS CONTAINING AZO DYE WASTEWATER

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Abstract

The effect of plant growth promoting rhizobacteria (PGPR) on seedling germination, plant growth and biomass yield of tomato plant in presence of dye wastewater irrigation was evaluated in 2 experiments (lab scale and green house). Previously isolated strain *Pseudomonas fluorescens* (Q14) with ACC deaminase activity was used to check its effect to remedify dye contaminated water impact on plants. The maximum biomass production was obtained on application of 600 mg/L dye with selected PGPR as compared to control water. The dye concentration beyond 600 mg/L showed significantly repressed growth of plants. In plant biomass analysis root and shoot biomass were measured while in plant growth root, shoot length in both experiments was compared. It was also observed that the inoculation with plant growth promoting bacteria carrying ACC deaminase activity promote root and shoot growth as compared to un-inoculated plants at different Reactive Black B concentrations. Maximum shoot (46 cm) and root length (35cm) was observed at 600 mg/L of Reactive Black B with inoculation, which clearly implies the importance of industrial wastewater for crop and vegetable grown under water stress conditions. These findings suggest that PGPR with ACC deaminase activity could be implied for increasing biomass production of wheat and maize irrigated with dye containing wastewater released from textile industries.

Introduction

Application of industrial wastewater for irrigation change the physicochemical characteristics of water bodies and soil and indirectly effect the growth of the plants (Zhang *et al.,* 2004). In textile industry upto 60 to 70% of all dyes applied are from azo dye group (-N=N). The release of azo dyes into the environment is a concern due to coloration of natural water bodies and toxicity to the plants and animals (Zollinger *et al*., 1987; Zhang *et al*., 2004; Hsueh *et al.,* 2009). Textile industries also use large volumes of water and various others chemicals for wet processing of textiles products. The chemicals are very much diverse in nature and chemically range from inorganic compounds to polymers and organic products (Banat *et al.,* 1996).

It is a common practice in water stressed countries to irrigate plants with grey water prior to discharge into water body coming from household or industrial sources (Ramana *et al.,* 2001). It solves the problem of water shortage, economically beneficial and can be disposed of during the entire year. Soil irrigated with grey water could have the properties like, decrease in soil pH, an increase in salt, organic matter content, CEC in the soil, and macro and micro nutrients concentrations (Chhonkar *et al.*, 2000). Because of its high nutritive value it may improve plant growth, reduce fertilizer application and increase productivity of poorly fertile soils (Munir *et al.,* 2007; Kiziloglu *et al.,* 2008). Application of industrial wastewater promotes germination but decreases plant survival as under chemical stress plant growth is stunted (Kaushik *et al*., 2005; Garg & Priya, 2006). Plant growth promoting bacteria are the vital solution to improve plant growth under chemical stress conditions.

Ethylene is a gaseous plant growth hormone produced endogenously by almost all plants. It is also produced in soil through a variety of biotic and abiotic mechanisms, and plays a key role in inducing multifarious physiological changes in plants at molecular level (Grichko & Glick, 2001; Azhar *et al.,* 2011). Apart from being a plant growth regulator, ethylene is also established stress hormone. Under stress conditions such as contaminants, salinity, drought, water logging, heavy metals and pathogenicity, the endogenous production of ethylene is accelerated substantially which adversely affects the root growth and consequently the growth of the plant as a whole. Certain plant growth promoting rhizobacteria (PGPR) contain a vital enzyme, 1-aminocyclopro-pane-1-carboxilate (ACC) deaminase, which regulates ethylene production by metabolizing ACC (an intermediate precursor of ethylene biosynthesis in higher plants) into α-ketobutyrate and ammonia. In this case ACC may also act as sole source of nitrogen for the PGPR (Shahzad *et al.,* 2008; Chookietwattana & Maneewan, 2012). Inoculation with PGPR containing ACC deaminase activity could be helpful in sustaining plant growth and development under stress conditions by reducing stress- induced ethylene production (Penrose & Glick, 2003; Mayak *et al.,* 2004, Naveed *et al.,* 2008; Kang *et al*., 2012).

It is very likely that dye contaminated textile water may cause accelerated synthesis of ethylene in plant tissues while PGPR containing ACC-deaminase may dilute the impact of contaminants by lowering ethylene levels. The present study was designed with the aim to study the effect of textile dye wastewater irrigation on tomato growth and to evaluate potential of plant growth promoting bacteria containing ACC-deaminase on tomato plant.

Materials and Methods

Soil physico-chemical analysis: The soil for this experiment was collected from upper 20 cm layer from a field area of Pir Mehr Ali Shah Arid Agriculture University Rawalpindi. The soil samples were air dried for one week and passed through 2 mm sieve before filling the pots. The soil was tested for its physico-chemical characteristics (Table 1). Soil texture was determined by hygrometric method.

Table 1. Physicochemical characteristics of soil

S. No	Parameter	Values
1.	pH	7.95 ± 0.05
2.	EC (dS m^{-1})	1.90 ± 0.04
3.	Soil texture	Clayey loam
4.	Particle size distribution (%)	
	Silt	12.0
	Sand	65.6
	Clay	22.4

Lab scale experiment: A four weeks lab experiment was conducted in glass jars (385 cm^3). The 36 plastic jars were filled with soil. The tomato seedling (*Solanum lycopersicum*) roots were disinfected by dipping them in 95% ethanol solution for 20 sec, following a dip in 0.2% (w/v) $HgCl_2$ solution for 30 sec as described by Khalid *et al.,* (2004). The treated seedlings were rinsed with sterilized water and inoculated with the inoculam of Q14 *Pseudomonas fluorescens* Q14 (Shaharoona *et al*., 2006) (OD 0.6 ± 0.02 at 600 nm) by dipping in culture medium for 10 minutes prior to transplant into jars. Similarly eighteen seedlings transplanted without dipping into bacterial culture. One time half strength of Hoagland nutrient solution was added to provide nutrients. Different concentrations (0, 200, 400, 600, 800, 1000 mg /L) of Reactive Black B were applied into jars after 2 days interval. Jars were kept in growth chamber and continuous light intensity of 800 µmol m^{-2} s^{-1} was given for 10 h per day. Control in both inoculated and uninoculated experiment with uncontaminated water application was kept for comparison.

Green house experiment: A three months pot experiment (36 pots) was done in green house. Pots were arranged into two sets containing eighteen pots in each set. In one set inoculation was done with strain Q14 and other was kept uninoculated. For inoculation seedlings were sterilized by dipping into 95% ethanol solution for 20 sec and in 0.2% (w/v) $HgCl_2$ solution for 30 sec. Than sterilized seedlings were dip into the culture medium containing Q14 strain (OD 0.6 ± 0.02 at 600 nm). Similarly other set seedlings were dip in same medium containing all the ingredients of culture except Q14. One time 10ml of half strength of Hoagland nutrient solution was added to provide nutrient. After inoculations seedlings were transplanted into pots and kept in green house. Reactive Black B solutions with the concentrations of 0, 200, 400, 600, 800, 1000 mg /L was applied after 2 days interval. The pots were arranged in a completely randomized design (CRD) with three replications for each treatment under green house conditions.

Observations taken: Weekly observations regarding plant height were taken in centimeter from glass jar experiment and fortnightly from pot experiment. The numbers of leaves were counted every 7 days interval in glass jar experiment. The numbers of branches were counted every 15 days interval in pot experiment. After harvesting of plants, their roots and shoots were separated and weighed by electrical balance. After weighing the roots and shoots were oven dried at 65˚C for 24 hours and weighed again. Fresh root and shoot weight and dry root and shoot weight of all plants were measured and compared.

Statistical analysis: The data were analyzed by using two factorial completely randomized designs. Means were calculated and individual means were compared by using LSD test at 5% level of significance. All the statistical analyses were performed by using M-STAT C software and Microsoft excel 2007.

Results and Discussions

A study was conducted to determine the impact of azo dye Reactive Black B applied in different concentrations and inoculation with PGPR on tomato plant growth under laboratory and green house conditions. It generally assumed that inoculation of the plant with PGPR containing acc-deaminase is helpful to dilute the impact of abiotic and biotic stresses. As the azo dyes are commonly used in different industries as colorant, which releases into environment along with industrial wastewater. In peri-urban areas this waste water commonly used to grow different vegetables.

Effect of rhizobacteria and reactive Black-B on root and shoot growth: There was a significant increase in both shoot and root length was observed with inoculated seedlings with Q14 at different concentrations of Reactive Black B as compared to uninoculated tomato seedlings. It was observed that initially with increasing dye concentration upto 600 mg/L, there was gradual increase in root and shoot length, whereas further increase in dye concentrations impacted severely root and there was a significantly decrease in length. Maximum root length was observed at 600 mg/L dye concentration with inoculated seedlings i.e., 10.6 cm as compared to uninoculated root length 9.9 cm (Fig. 1), while further increase in Reactive Black B there is significant decrease in root length was observed and lowest (0.8 cm) was observed at 1000 mg/L of Reactive Black B concentration (Fig. 1). Similarly in case of shoot length there was 9% increase was observed at 600 mg/L dye concentration after 28 days (Fig. 2). However no significant difference was found in shoot length between inoculated and un-inoculated plants after 90 days of experiment in green house experiment (Fig. 3). Maximum shoot length in case of green house experiment was 47cm, which is non significantly higher than the uninoculated seedling. But it was observed that inoculated plants had better look in withstand and appearance. The increased in root and shoot length was probably due to the carbon and nitrogen present in dyes (Mahmood *et al.*, 2011), which play an important role in plant growth. Similarly better growth in inoculated plants can be attributed to the acc deaminase activity of the Q14 (PGPR), as it was assumed that in any plant stress (biotic and abiotic) it tend to produce higher level of ethylene (Penrose & Glick, 2003; Mayak *et al.,* 2004) which indirectly reduce the plant growth. The use of plant growth promoting bacteria with acc deaminase activity decreased the damage to plants (Mayak *et al.,* 2004). The presence of rhizobacterial strains in roots could decreased the level of ethylene in root tissues by metabolizing ACC into α-ketobutyrate and ammonia in presence of aminocyclopropane-1-carboxilate (ACC) deaminase enzyme and in turn promote root and shoot growth due to the ACC deaminase activity (Glick *et al.*, 1998).

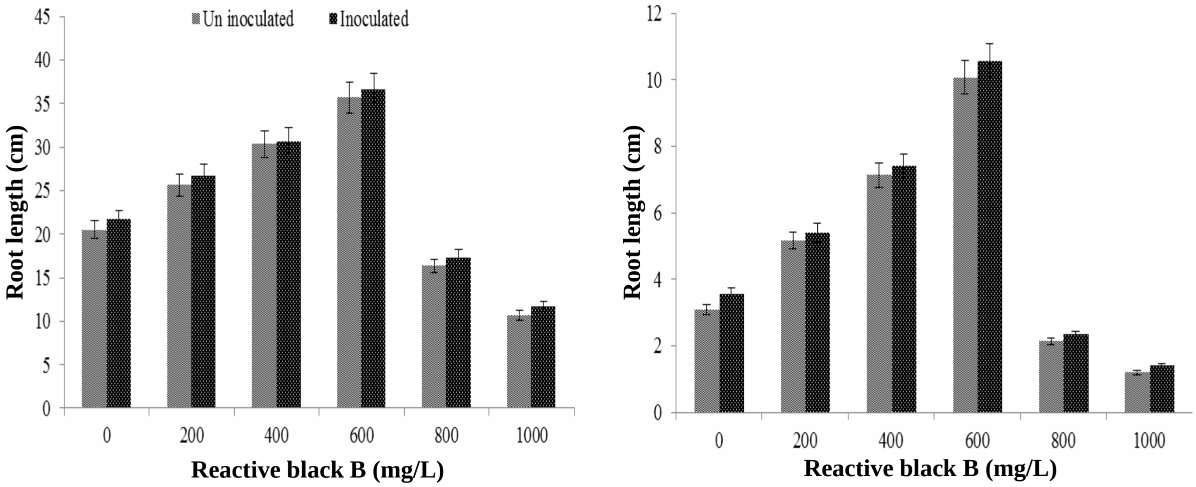

Fig. 1. The effect of different concentration of Reactive Black B on root length of tomato seedlings with or without inoculation with Q14 strain in jar (a) and pot experiments (b).

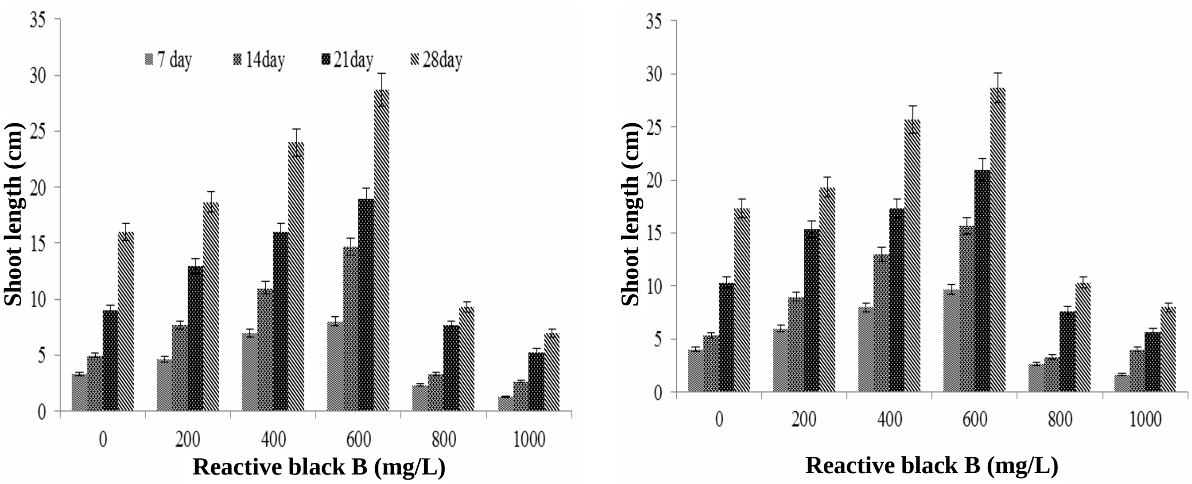

Fig. 2. The effect of different concentration of Reactive Black B on shoot length of tomato seedlings with (b) or without (a) inoculation with Q14 strain after different time periods in jar experiment (Lab conditions)

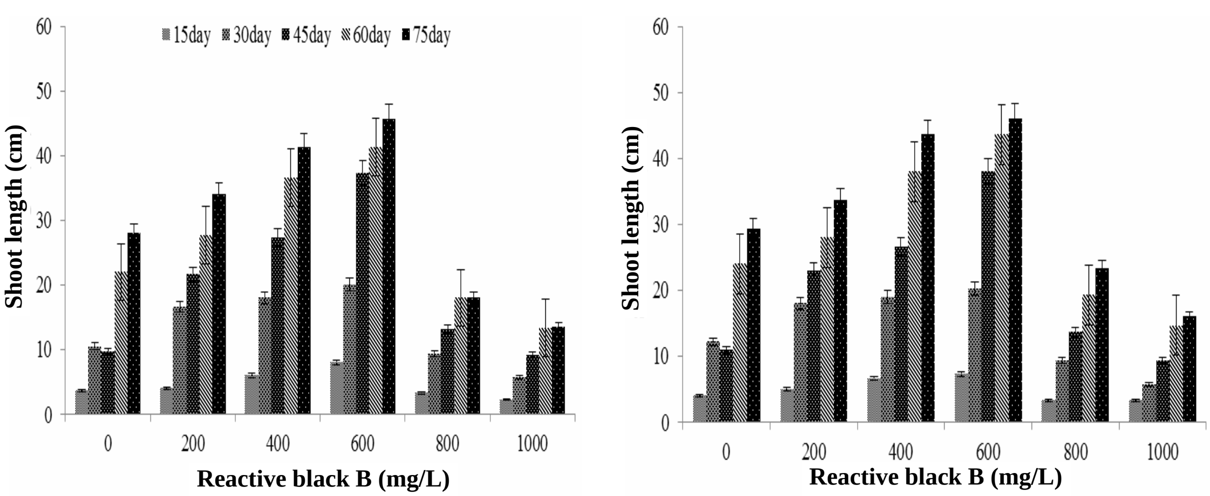

Fig. 3. The effect of different concentration of Reactive Black B on shoot length of tomato seedlings with (b) or without (a) inoculation with Q14 strain after different time periods in pot experiment (Green house conditions).

Root and shoot biomass: Similarly root and shoot biomass was taken after the harvest of the both experiments (Jar and green house). Maximum shoot biomass was observed at 600 mg/L of dye concentration with and without Q14 inoculation 1.1 g/plant (green house experiment) and 0.87 g/plant (jar experiment) respectively (Figs. 4 & 5). Root biomass was also maximum again at same concentration of the dye (0.36 g/plant). Initially a gradual increase in both root and shoot biomass was observed up to the 600 mg/L concentration of Reactive Black B, but further increase in dye concentration a sharp decline in root and shoot biomass was recorded. However at maximum dye concentration 1000 mg/L the impact was more sever on root biomass, where it was reduced to only 0.02 g/plant (Fig. 5) as compared to shoot biomass which was 0.2 g/plant at same dye concentration (Fig. 4). At 600 mg/L Reactive Black B about 150 and 120% increase was found in root and shoot biomass respectively as compared to non contaminated water, which clearly implies the importance of this wastewater for crop grown. This increase in biomass at reasonably higher concentration of dye may be due to the activated synthesis of plant growth hormones to trigger the activity of specific enzymes, which can brought an increase in availability of starch assimilation in plants resulting in increased biomass production (Arshad *et al.,* 2008).

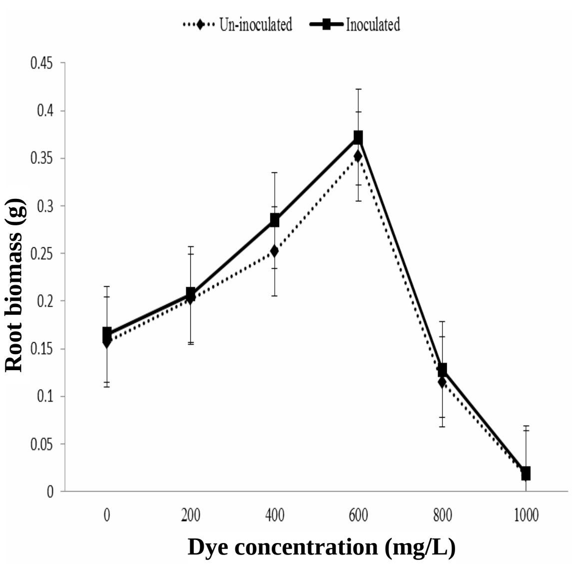

Fig. 4. The effect of different concentration of Reactive Black B on root biomass of tomato seedlings with or without inoculation with Q14 strain in jar experiment (Lab conditions).

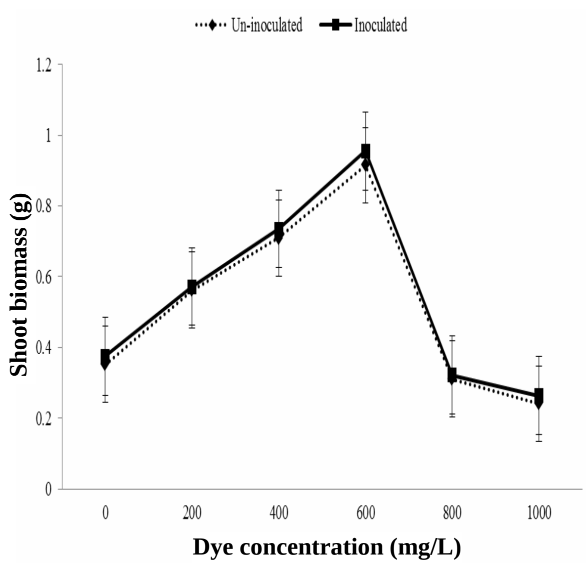

Fig. 5. The effect of different concentration of Reactive Black B on root biomass of tomato seedlings with or without inoculation with Q14 strain in pot experiment (Green house conditions).

Number of leaves and branches: Another important parameter which can be affected by the dye contaminated water was the number of leaves and branching of the plant. These parameters were only measured in pot trial as it had no significant in short span experiment. In this experiment, the number of leaves of the tomato plant was increased when treated with dye concentrations of 600 mg/L but further increase of dye concentrations i.e., 1000 mg/L, the leave number start to decrease (Fig. 6). Similarly leave fall is more sever under higher dye a concentration that clearly indicates the higher concentrations of ethylene produced by the plant under dye contaminated water stress. Similarly with the leave number shoot branches were reduced in both above and below the 600 mg/L dye concentration in all observations taken after 30, 45, 60, 75 and 90 days (Fig. 7). However inoculation effect was minimum in this case and variation in results was totally due to change in dye concentrations. The increase in number of the leaves may be due to the presence of organic medium and microorganisms as both the organic medium and microorganisms support plant growth and number of leaves (Gravel *et al*., 2007). Similarly reduction of the growth at higher dye concentration may be due to the toxicity of this dye at increasing dye levels. Similarly at 0 mg/L dye the impact of inoculation with Q14 was more prominent, which indicates the toxicity of the dye for PGPR as well at higher dye concentrations (Mahmood *et al*., 2011). In general present study will be very helpful to establish the role of PGPRs in stress tolerance of plant under higher concentrations of dye in peri-urban areas to grow vegetables and crops.

Conclusion

The results of this study clearly indicates that up to certain limits dye concentrations in wastewater can be supportive to the plant growth and furthermore dye contaminated wastewater impact can be diluted with the application of PGPR with acc deaminase activity. However further studies are needed in order to assess the uptake of dyes by the plant and its role in overall quality of the final plant product.

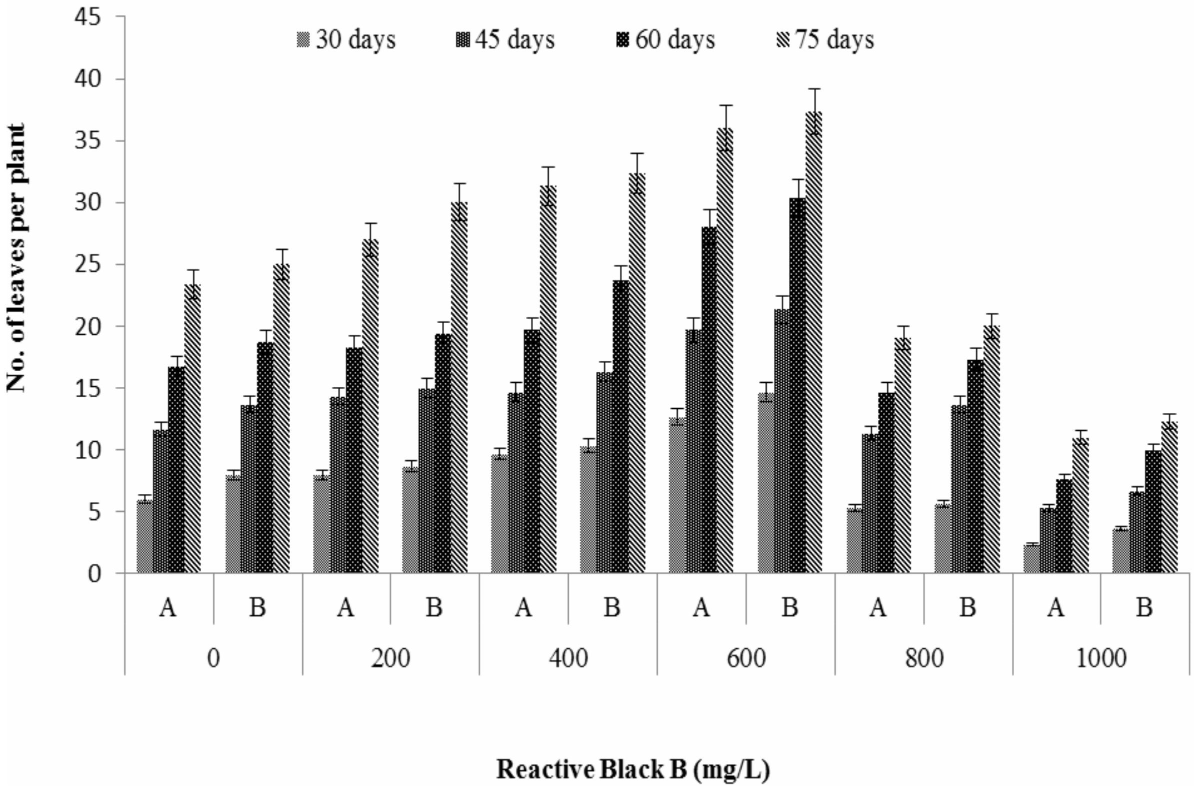

Fig. 6. The effect of different concentration of Reactive Black B on number of leaves per plant of tomato seedlings with (B) or without (A) inoculation with Q14 strain in pot experiment (Green house conditions).

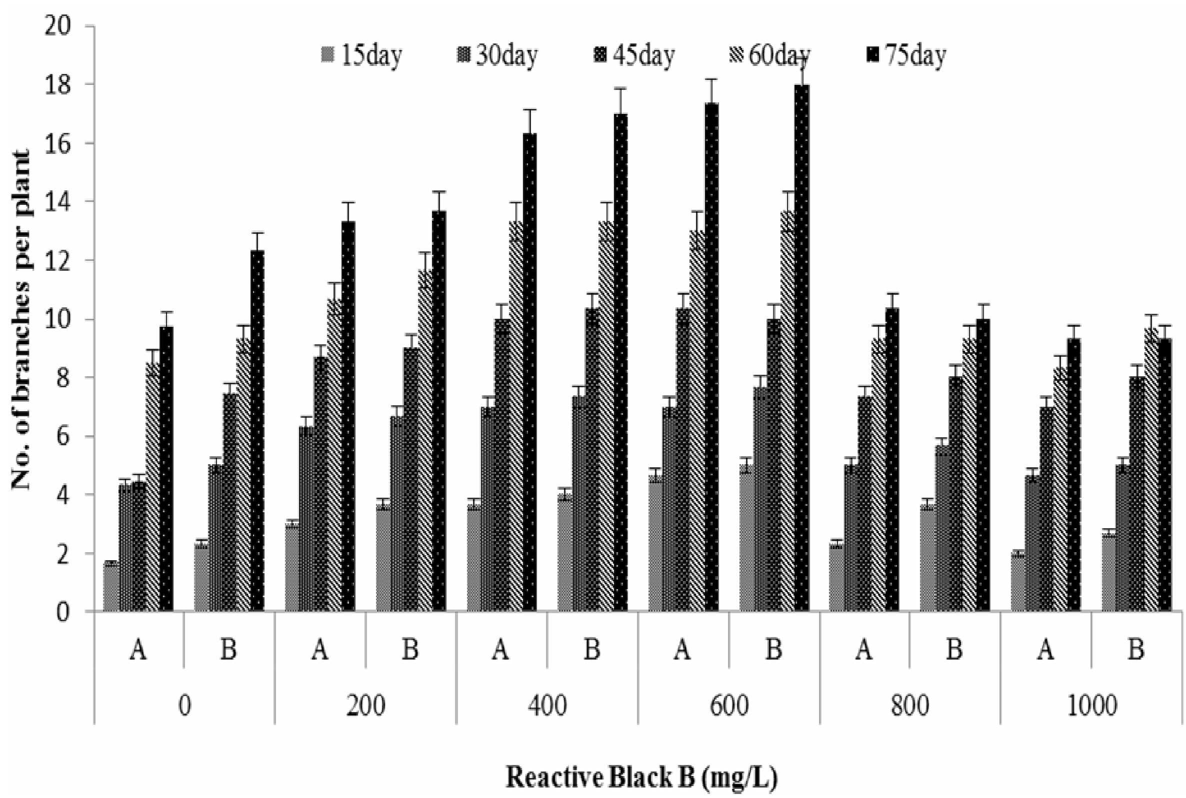

Fig. 7. The effect of different concentration of Reactive Black B on number of branches per plant of tomato seedlings with (B) or without (A) inoculation with Q14 strain in pot experiment (Green house conditions).

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(Received for publication 1 September 2012)