Effect of seed inoculation and foliar application of biofertilizers on some biochemical and morphological characteristics of waterlogged-canola

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Abstract: Waterlogging stress restricts growth and yield of canola by undesirable physiological changes. Thus, the aim of this study was to compare the influences of the seed inoculation and foliar application of two biofertilizers on selected biochemical and morphological characteristics of canola plants (*Brassica napus* L. cv. Hayola 401) under the waterlogging stress conditions. Two biofertilizers, including AAP (*Azotobacter chroococcum, Azospirillum spp.* and *Pseudomonas spp.*) and APB (*Azospirillum spp.*, *Pseudomonas fluorescens* and *Basillus subitilis*), were used as seed inoculation or foliar spray at different times on waterlogged seedlings of canola (at 5-leaf growth stage). The data analysis showed that the content of chlorophyll *a*, chlorophyll *b*, chlorophyll *a*/*b* ratio and carotenoid in the leaves of canola were reduced by this stress. The number of siliques per plant, grain yield, plant height, stem width, number of branches and branching position also significantly decreased as a result of the waterlogging stress. The application of biofertilizers either by seed inoculation or foliar sprays significantly alleviated the waterlogging effects. It was evinced by the higher pigments content in the leaves, the increase in the number of siliques per plant, the greater grain yield and increase in the other morphological characteristics over the waterlogged control. However, we concluded that, among the applied methods, inoculating the seeds with the biofertilizers is advisable to alleviate the waterlogging damage in canola.
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1. Introduction

One of the world's major oilseed crops is canola (*Brassica napus* L.). It is the most important source of edible oil. Flooding or an excess of water availability is an important global crop production constraint and causes significant yield reductions in canola (Zhou, 1994). Such yield reductions may occur after 3 to 30 days of flooding stress, depending on the climate and the developmental stage of the plants (Gutierrez Boem et al., 1996). Zhou and Lin (1995) reported that the physiological reactions to waterlogging at the seedling and floral bud appearance stages of canola were associated with decreases in the leaf chlorophyll content, decrese in the antioxidant enzymatic activities, the plant height, the accumulation of leaf malondialdehyde, a greater ethylene production, and a reduction in the leaf photosynthetic rate. A lack of O_2 due to waterlogging may limit the crop growth because of alterations in metabolism (Drew, 1992) and the nutrient uptake of plants, leading to the generation of active oxygen species. These toxic oxygen species react with numerous cell components and cause oxidative stress (Scandalios, 1993). Plants may respond to waterlogging by altering their hormone balance and the growth of stems and roots (Grichko and Glick, 2001b); indeed, the ethylene production in shoots is responsible for the abnormal growth of plants under waterlogged conditions (Saleem et al., 2007).

Nutrient deficiency is the major cause for the poor plant growth in waterlogged soils (Steffens et al., 2005). Nitrogen deficiency may be induced by the low redox potential in waterlogged soils that promotes denitrification of nitrate anions. The anaerobic condition inhibits the root metabolism and root growth (Drew, 1992). Gutierrez Boem et al. (1996) reported that waterlogging resulted in a decrease of nitrogen (N) uptake and other macronutrients by canola.

Seed inoculation and the foliar spray of biofertilizers (products containing plant growth-promoting rhizobacteria [PGPR]) have been used for boosting plant growth and reducing the negative effects of stress conditions (Wu et al., 2005; Saleem et al., 2007). Basha et al. (2006) found that the application of PGPR as a foliar spray provided a superior efficiency in the management of fungal diseases on chickpea, and Esitken et al. (2006) reported a significant effect of a PGPR foliar spray in increasing the yield of sweet cherry. Vijayan et al.

(2007) concluded the better performance of a foliar application of *Azotobacter chroococcum* in alleviating the growth-inhibiting effects of salinity in mulberry plants, and Grichko and Glick (2001a) reported that tomato plants inoculated with PGPR showed a substantial tolerance to waterlogging stress. However, no research has been conducted thus far to compare the effects of foliar and seed applications of PGPR on canola under waterlogged stress conditions. Therefore, the objective of this work was to compare the effects of foliar and seed applications of two biofertilizers on selected biochemical and morphological characteristics of canola plants subjected to waterlogging stress.

2. Material and Methods

The experiment was conducted for two years, during the winter of 2010 and 2011, in a greenhouse at Sari Agricultural Sciences and Natural Resources University (53º 13′ E and 36º 42′ N), Sari, Mazandaran Province, Iran. The experiment was arranged in a completely randomized design with 10 treatments, and each treatment was replicated three times. The seeds of canola (*Brassica napus* L.) cv. Hayola 401 were planted in plastic pots (37 cm diameter and 45 cm depth) containing approximately 35 kg of clay loam soil (36 % clay, 41 % silt and 23 % sand). Ten seeds were planted in each pot, and after full germination, the number of plants was reduced to four seedlings per pot. The plants were irrigated at the field capacity level.

The treatments included waterlogged (WL) and non-waterlogged (NWL) controls. Four levels of biofertilizer applications included the following: seed inoculation; foliar spray before waterlogging (FSBW) at the 3-leaf growth stage; foliar spray after waterlogging (FSAW) and foliar spray before and after waterlogging (FSBAW). Two biofertilizers were used, as follows: AAP (*Azotobacter chroococcum*, *Azospirillum brasilense*, *A. lipoferum*, *Pseudomonas fluorescens* and *P. putida*) and APB (*Azospirillum brasilense*, *A. lipoferum*, *Pseudomonas fluorescens* and *Basillus subitilis*) (Asia Biotechnology Institute, Tehran, Iran). The bacterial concentration of each biofertilizer was 10^8 CFU ml^{-1}.

All of the pots (except for the NWL control) were uniformly subjected to waterlogging stress at the 5-leaf growth stage for two weeks. To apply the waterlogging treatments, each pot was placed into a plastic bucket (40 cm diameter and 48 cm depth). The waterlogging treatments were then applied by filling the outer container with water up to 2 cm above the soil surface.

The photosynthetic pigments including chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoid were measured in fresh leaf samples, two weeks after the end of waterlogging stress according to the method described by Lichtenthaler, 1987. In brief, the leaf samples (0.1 g) were homogenized with acetone (80% v/v), filtered and make up to a final volume of 20 ml and the pigment concentrations were calculated from the absorbance of extract at 663.2, 646.8 and 470 nm using a Biowave II spectrophotometer (Biochrom Ltd., Cambridge, UK) by the formula given below:

Chlorophyll *a* (µg ml^{-1}) = (12.25 $A_{663.2}$ – 2.79 $A_{646.8}$)

Chlorophyll *b* (µg ml^{-1}) = (21.50 $A_{646.8}$ –5.10 $A_{663.2}$)

Carotenoid (µg ml^{-1}) = (1000 A_{470} – 1.82Chl*a*– 85.02Chl*b*)/198

The leaf pigments concentration was expressed as the mg g^{-1} FW.

Plant height (cm), stem width (cm), number of branches and branching position (cm) were measured from all plants at harvest. Number of grains per silique, number of siliques per plant and the 1000-seed weight (g) were measured and the grain yield (g $plant^{-1}$) was obtained as aggregate of all yield components.

A Bartlett's test of homogeneity of variance was performed on all parameters among years. This test showed the homogeneity for all parameters, so years were pooled. All of the data in the present study were subjected to an analysis of variance (ANOVA). The means were separated by LSD test using SAS software.

3. Results

The experimental treatments had a significant effect on chlorophyll *a*, chlorophyll *b*, chlorophyll *a*/*b* ratio and carotenoid content of the canola leaves ($p < 0.01$) as shown in Table 1. The number of siliques per plant ($p < 0.01$), grain yield ($p < 0.05$), plant height, stem width, number of branches and branching position ($p < 0.01$) also significantly influenced by applied treatments (Table 3).

Waterlogging stress significantly decreased the content of chlorophyll *a*, chlorophyll *b*, chlorophyll *a*/*b* ratio and carotenoid in the leaves of canola plants compared with the non-waterlogged control (Table 2).

The content of chlorophyll *a* in the canola leaves was increased by both biofertilizers and methods of application compared to the WL control. There was not any significant difference between two biofertilizers. The seed inoculation (especially for AAP biofertilizer), FSBW and FSBAW of biofertilizers were slightly superior than the FSAW of biofertilizers regarding to the increase the content of chlorophyll *a* in the canola leaves (Table 2).

Table 1. The mean squares of ANOVA for the chlorophyll *a* content (mg g^{-1} FW), chlorophyll *b* content (mg g^{-1} FW), chlorophyll *a*/*b* ratio and carotenoid content of canola leaves (mg g^{-1} FW); the number of grains per silique and 1000-grain weight (g) of canola plants.

S.O.V.	df	Chlorophyll *a* content (mg g^{-1} FW)	Chlorophyll *b* content (mg g^{-1} FW)	Chlorophyll *a*/*b* ratio	Carotenoid content (mg g^{-1} FW)	Number of grains per silique	1000-grain weight (g)
Y	1	0.00003 ns	0.00001 ns	0.00002 ns	0.00130 ns	0.0167 ns	0.00001 ns
R×Y (E_a)	4	0.00034	0.00005	0.00237	0.00069	0.1667	0.00875
T	9	0.02540 **	0.00224 **	0.01117 **	0.00201 **	0.6092 ns	0.00463 ns
T × Y	9	0.00248 ns	0.00031 ns	0.00004 ns	0.00017 ns	0.4611 ns	0.01779 ns
E_b	36	0.00089	0.00017	0.00123	0.00026	0.8518	0.02487
CV%		3.22463	3.86717	1.28858	5.45012	5.1228	4.63899

Note. ** – $p < 0.01$, ns – $p > 0.05$. Y– Year, R – Replication, T – Treatment

Table 2. The effect of experimental treatments on the chlorophyll *a* content (mg g^{-1} FW), chlorophyll *b* content (mg g^{-1} FW), chlorophyll *a*/*b* ratio, carotenoid content (mg g^{-1} FW) of canola leaves; number of grains per silique and 1000-grain weight (g) of canola plants.

Treatments		Chlorophyll *a* content (mg g^{-1} FW)	Chlorophyll *b* content (mg g^{-1} FW)	Chlorophyll *a*/*b* ratio	Carotenoid content (mg g^{-1} FW)	Number of grains per silique	1000-grain weight (g)
Controls	Non-waterlogged	1.075 a	0.383 a	2.807 a	0.340 a	17.83 ab	3.44 a
	Waterlogged	0.818 g	0.310 e	2.636 d	0.283 d	18.00 ab	3.38 a
Seed inoculation	AAP Biofertilizer	0.953 b	0.351 b	2.715 bc	0.313 b	18.67 a	3.36 a
	APB Biofertilizer	0.942 bc	0.343 bc	2.741 bc	0.310 b	18.00 ab	3.40 a
Foliar spray before waterlogging	AAP Biofertilizer	0.932 bcd	0.344 bc	2.711 bc	0.295 bcd	17.83 ab	3.36 a
	APB Biofertilizer	0.912 cdef	0.332 cd	2.750 b	0.290 cd	18.33 ab	3.40 a
Foliar spray after waterlogging	AAP Biofertilizer	0.897 ef	0.331 cd	2.703 c	0.282 cd	17.50 b	3.43 a
	APB Biofertilizer	0.833 f	0.323 de	2.735 bc	0.280 d	17.83 ab	3.42 a
Foliar spray before and after waterlogging	AAP Biofertilizer	0.923 bcde	0.340 bc	2.713 bc	0.297 bcd	18.00 ab	3.39 a
	APB Biofertilizer	0.905 def	0.330 cd	2.740 bc	0.300 bc	18.17 ab	3.41 a
LSD ($p < 0.05$)		0.035	0.015	0.041	0.019	1.08	0.185

Means not sharing a common letter in a column differ significantly at 0.05 level of probability.

Table 3. The mean squares of ANOVA for the number of siliques per plant, grain yield (g $plant^{-1}$), plant height (cm), Number of branches and branching position (cm) of canola plants.

S.O.V.	df	Number of siliques per plant	Grain yield (g $plant^{-1}$)	Plant height (cm)	Stem width (cm)	Number of branches	Branching position (cm)
Y	1	0.070 ns	0.0075 ns	2.562 ns	0.0004	0.040 ns	0.461 ns
R×Y (E_a)	4	15.783	0.1217	11.812	0.0003	0.010	9.941
T	9	224.602 **	0.9741 *	144.469 **	0.0136 **	0.149 **	54.802 **
T × Y	9	23.970 ns	0.0985 ns	0.668 ns	0.0020	0.007 ns	0.753 ns
E_b	36	56.621	0.4174	7.477	0.0008	0.013	6.091
CV%		6.919	9.7081	2.398	3.8924	2.979	4.098

Note. * – $p < 0.05$, ** – $p < 0.01$, ns – $p > 0.05$. Y– Year, R – Replication, T – Treatment

Table 4. The effect of experimental treatments on the number of siliques per plant, grain yield (g plant^{-1}), plant height (cm), stem width, number of branches and branching position (cm) of canola plants.

Treatments		Number of siliques per plant	Grain yield (g plant^{-1})	Plant height (cm)	Stem width (cm)	Number of branches	Branching position (cm)
Controls	Non-waterlogged	121.80 a	7.47 a	122.37 a	0.863 a	4.24 a	67.66 a
	Waterlogged	96.89 c	5.91 c	102.17 c	0.681 c	3.65 c	56.68 e
Seed inoculation	AAP Biofertilizer	110.54 b	6.92 ab	113.28 b	0.717 b	3.80 b	59.81 bcd
	APB Biofertilizer	111.22 b	6.79 ab	114.43 b	0.727 b	3.78 bc	61.02 bc
Foliar spray before waterlogging	AAP Biofertilizer	107.81 b	6.45 bc	114.35 b	0.723 b	3.78 bc	58.22 cde
	APB Biofertilizer	106.78 b	6.66 bc	113.70 b	0.730 b	3.84 b	59.37 bcde
Foliar spray after waterlogging	AAP Biofertilizer	106.78 b	6.42 bc	114.78 b	0.720 b	3.75 bc	59.53 bcde
	APB Biofertilizer	106.10 b	6.48 bc	113.98 b	0.722 b	3.72 bc	57.67 de
Foliar spray before and after waterlogging	AAP Biofertilizer	108.83 b	6.64 bc	116.12 b	0.726 b	3.84 b	60.97 bc
	APB Biofertilizer	109.86 b	6.79 ab	114.82 b	0.723 b	3.78 bc	61.30 b
LSD ($p < 0.05$)		8.803	0.757	3.202	0.033	0.13	2.89

Means not sharing a common letter in a column differ significantly at 0.05 level of probability.

The seed inoculation or foliar spray of both biofertilizers significantly increased the content of chlorophyll *b* in the canola leaves compared to the WL control. The effect of seed inoculation of AAP biofertilizer was higher pronounced than the other treatments (Table 2).

The chlorophyll *a*/*b* ratio in the canola leaves was significantly increased by seed inoculation or all foliar applications compared to the WL control (Table 2). The higher increasing effect was obtained by FSBW of APB biofertilizer, while the less effect was related to the FSAW of AAP biofertilizer.

The content of carotenoid in the leaves of canola plants was also significantly increased by seed inoculation of both biofertilizers. Among the foliar applications, the FSBAW of APB biofertilizer also significantly increased the carotenoid content in the canola leaves compared to the WL control (Table 2).

The waterlogging stress caused a significant decrease in the number of siliques per plant, grain yield, plant height, stem width, number of branches and branching position of canola plants compared with the non-waterlogged (NWL) control (Table 4).

The seed inoculation or foliar spray of both biofertilizers at any times of application significantly increased the number of siliques per plant compared to the waterlogged control. There was not any significant difference between the various applications of both biofertilizers. This means that, they had a similar effect on increase the number of siliques per plant (Table 4).

The seed inoculation of both biofertilizers and FSBAW of APB biofertilizer significantly increased the grain yield compared to the WL control, without showing any significant difference from NWL control. The non-significant effects on increase the grain yield compared to WL control were similar between the other foliar applications (Table 4).

The plant height and stem width were significantly increased either by seed inoculation or foliar applications of both biofertilizers compared to the WL control, though there was not any significant difference among them (Table 4).

The seed inoculation of AAP biofertilizer, FSBAW of APB biofertilizer and FSBAW of AAP biofertilizer significantly increased the number of branches compared to the waterlogged control. The other applications had not a significant effect on increase the number of branches compared to the WL control (Table 4).

The branching position significantly increased due to the seed inoculation or FSBAW of both biofertilizers, compared with the WL control. The effects of the other applications on increase the

branching position compared to the WL control were not significant (Table 4).

4. Discussions

Different genera such as Azoarcus, Azospirillum, Azotobacter, Arthrobacter, Bacillus, Clostridium, Enterobacter, Gluconoacetobacter, Pseudomonas, and Serratia have been used as PGPR (Jaleel et al., 2009; Somers et al., 2004). However among these different PGPR, genera such as Azotobacter, Azospirillum, Psuedomonas and Bacillus are widely used as biofertilizers in the field of agriculture. Performance of biofertilizers could be explained by the fixation of sufficient atmospheric nitrogen, production of plant growth promoters, decreasing the ethylene production in plants and solubilization of minerals such as phosphorus (Vessey, 2003; Karthikeyan et al., 2008a, b).

The results of our experiment provide a new proof in the clarification of the mechanism that underlies the ability of biofertilizers to help plants tolerate waterlogging damage. The Nitrogen fixing is one of the most important effects of PGPR under waterlogging stress which encounters plants with the nitrogen deficiency (Gutierrez Boem et al., 1996). Nitrogen has a role in the synthesis of cytokinins, which is important in chlorophyll synthesis and retarding the leaf senescence (Argueso et al., 2009). Therefore, nitrogen deficiency induces leaf senescence in plants provoked by lipid peroxidation and pigment loss as well as protein degradation that leads to the inhibition of photosynthetic capacity (Casano et al., 1994). Thus, the effect of biofertilizers in this experiment can be justified, at least partly, to the ability of PGPR to fixing nitrogen.

It is known that the leaf senescence is regulated by several factors including genetic, hormonal, and environmental signals or stresses (Liu et al., 2010). The hypothesis of the research was that the yield reduction under waterlogging stress condition is a result of inhibition in the photosynthetic apparatus which is caused by leaf senescence. The decrease in chlorophyll *a* and *b* and ratio of chlorophyll *a*/*b* due to the waterlogging stress manifested the higher rate of chlorophyll *a* degradation than chlorophyll *b*. In the present experiment there was a strong correlation between the grain yield and the content of chlorophyll *a* and *b* in leaves (r = 0.904** and 0.902**, respectively). However, the increase in the chlorophyll contents and reduction in the leaf senescence by biofertilizers application, conform the hypothesis of the research. Moreover, the morphological characteristics of canola seedlings (plant height, stem width, branch number and position) were significantly increased either by seed inoculation or foliar application of biofertilizers.

The overproduction of ethylene in response to abiotic and biotic stresses leads to the inhibition of root growth and, consequently, the inhibition of the growth of the entire plant (Bleecker and Kende, 2000). Leul and Zhou (1998) observed an increase in the level of ethylene production in the leaves of canola under waterlogging stress. It has been proposed that the PGPR can lower the ethylene levels and relieving negative effect of this gaseous hormone on plant growth (Ma et al., 2002). Two mechanisms, including rhizobitoxine excretion and ACC deaminase activity, for the role of PGPR in lowering ethylene levels have been proposed. Rhizobitoxine is a toxin that inhibits ACS activity (Yuhashi et al., 2000), and the synthesis of rhizobitoxine has been identified in only in a few bacteria belonging to the Bradyrhizobium (Yasuta et al., 2001) and the Pseudomonas genera (Mitchell and Coddington, 1991). ACC deaminase (AcdS, EC: 3.5.99.7) catalyzes the degradation of ACC into α-ketobutyrate and ammonia. The PGPR that express ACC deaminase regulate and lower the levels of ethylene. These ACC deaminase-producing PGPR boost plant growth, particularly under the conditions of stress, by the regulation of accelerated ethylene production in response to a multitude of abiotic and biotic stresses, such as salinity, drought, waterlogging, temperature, pathogenicity and contaminants (Hontzeas et al., 2005). Moreover, the ability of many rhizobacteria to produce plant hormones or hormone-like substances has often been evoked to explain how PGPR can promote plant growth (Bloemberg and Lugtenberg, 2001; Jaleel et al., 2007).

The application of both biofertilizers via any of various applications methods (seed inoculation and foliar application) significantly alleviated the growth-inhibiting effects of waterlogging stress. The increase in grain yield confirms this effect. The number of siliques per plant was responsible for the significantly greater grain yield over the waterlogged control. The positive correlation between the number of siliques per plant and the grain yield (r = 0.972**) also confirms this influence. The positive effects of PGPR inoculation on plant growth were confirmed by various researches (Ibiene et al., 2012). Grichko and Glick (2001a) reported a positive effect of PGPR inoculation on waterlogged-tomato plants. Vijayan et al., (2007) also demonstrated the beneficial effect of foliar application of *Azotobacter chroococcum* on mulberry under salinity stress condition.

The current study is the first evaluation regarding the comparison of the effect of the seed inoculation and foliar application of biofertilizers on waterlogging tolerance in canola. The application of

biofertilizers by both tested methods significantly alleviated the growth-inhibiting effects of the waterlogging stress. Despite the similar effect of both application methods, it may be concluded that the seed inoculation of the biofertilizer is the advisable method to enhance the tolerance to flooding stress in canola. Seed inoculation of biofertilizers is easier to use and more cost efficient than their foliar application.

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References

1. Argueso CT, Ferreira FJ, Kieber JJ. Environmental perception avenues: the interaction of cytokinin and environmental response pathways. Plant Cell Environ. 2009; 32: 1147–1160.
2. Basha SA, Sarma BK, Singh DP, Annapurna K, Singh UP. Differential methods of plant growth-promoting rhizobacteria induce synthesis of phenylalanine ammonia-lyase and phenolic compounts differentially in chickpea. Folia microbiol. 2006; 51: 463–468.
3. Bleecker AB, Kende H. Ethylene: a gaseous signal molecule in plants. Annu. Rev. Cell Dev. Biol. 2000; 16: 1–18.
4. Bloemberg GV, Lugtenberg BJJ. Molecular basis of plant growth promotion and biocontrol by rhizobia. Curr. Opin. Plant Biol. 2001; 4: 343–350.
5. Casano, LM, Martin M, Sabater B. Sensitivity of superoxide dismutase transcript levels and activities of oxidative stress is lower in mature-senescent than in young barley leaves. Plant Physiol. 1994; 106: 1033–1039.
6. Drew MC. Soil aeration and plant root metabolism. Soil Sci. 1992; 154: 259-268.
7. Esitken A, Pirlak L, Turan M, Sahin F. Effects of floral and foliar application of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrition of sweet cherry. Scientia Hortic. 2006; 110: 324–327.
8. Grichko VP, Glick BR. Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. Plant Physiol. Biochem. 2001a; 39:11–17
9. Grichko VP, Glick BR. Ethylene and flooding stress in plants. Plant. Physiol. Biochem. 2001b; 39:1-9.
10. Gutierrez Boem FH, Lavado RS, Porcelli CA. Note on the effects of winter and spring waterlogging on growth, chemical composition and yield of rapeseed. Field Crops Res. 1996; 47: 175–179.
11. Hontzeas N, Richardson AO, Belimov A, Safronova V, Abu-Omar MM, Glick BR. Evidence for horizontal transfer of 1-aminocyclopropane-1-carboxylate deaminase genes. Appl. Environ. Microbiol. 2005; 71: 7556–7558.
12. Ibiene AA, Agogbua JU, Okonko IO and Nwachi GN. Plant growth promoting rhizobacteria (PGPR) as biofertilizer: Effect on growth of Lycopersicum esculentus. J. Am. Sci. 2012; 8:318-324.
13. Jaleel, CA, Manivannan P, Sankar B, Kishorekumar A, Gopi R, Somasundaram R. Panneerselvam R. Pseudomonas fluorescens Enhances Biomass Yield and Ajmalicine Production in Catharanthus roseus under Water Deficit Stress. Colloids Surf. B: Biointerf. 2007; 60: 7-11.
14. Jaleel, C. A., R. Gopi, M. Gomathinayagam, and R. Panneerselvam. Traditional and Nontraditional Plant Growth Regulators Alters Phytochemical Constituents in Catharanthus roseus. Process Biochem. 2009; 44: 205–209.
15. Karthikeyan, B, Jaleel CA, Zhao CX, Joe MM, Srimannarayan J, Deiveekasundaram M. AM Fungi and Phosphorus Levels Enhances the Biomass Yield and Ajmalicine Production in Catharanthus roseus. EurAsia J. Biosci. 2008a; 2: 26–33.
16. Karthikeyan B, Jaleel CA, Lakshmanan GMA, Deiveekasundaram M. Studies on Rhizosphere Microbial Diversity of Some Commercially Important Medicinal Plants, Colloids Surf. B: Biointerf. 2008b; 62: 143–145.
17. Leul M, Zhou W. Alleviation of waterlogging damage in winter rape by application of uniconazole, Effects on morphological characteristics, hormones and photosynthesis. Field Crops Res. 1998; 59: 121-127.
18. Lichtenthaler HK. Chlorophylls and carotenoid: pigments of photosynthetic biomemranes. Methods Enzymol. 1987; 148:350–382.
19. Liu X, Li Z, Jiang Z, Zhao Y, Peng J, Jin J, Guo H, Luo J. LSD: a leaf senescence database. Nucleic Acids Res. 2010; 1-5.

20. Ma W, Penrose DM, Glick BR. Strategies used by rhizobia to lower plant ethylene levels and increase nodulation. Can. J. Microbiol. 2002; 48: 947–954.
21. Mitchell RE, Coddington JM. Biosynthetic pathway to rhizobitoxine in *Pseudomonas androponis*. Phytochemistry. 1991; 30: 1809–1814.
22. Saleem M, Arshad M, Hussain S, Bhatti AS. Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J. Ind. Microbiol. Biotechnol. 2007; 34: 635–648.
23. Scandalios JG. Oxygen stress and superoxide dismutases. Plant Physiol. 1993; 101: 7–12.
24. Somers E, Vanderleyden J, Srinivasan M. Rhizosphere Bacterial Signalling: A Love Parade beneath Our Feet. Crit. Rev. Microbiol. 2004; 30: 205–240.
25. Steffens D, Hütsch BW, Eschholz T, Lošák T, Schubert S. Waterlogging may inhibit plant growth primarily by nutrient deficiency rather than nutrient toxicity. Plant Soil Environ. 2005; 51: 545–552.
26. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil. 2003; 255: 571–586.
27. Vijayan K, Chakraborti SP, Ghosh PD. Foliar application of *Azatobactor chroococcum* increases leaf yield under saline conditions in mulberry (*Morus spp.*). Scientia Horticulturae. 2007; 113: 307–311.
28. Wu SC, Cao ZH, Li ZG, Cheung KC, Wong MH. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma. 2005; 125: 155–166.
29. Yasuta T, Okazaki S, Mitsui H, Yuhashi K, Ezura H, Minamisawa K. DNA sequence and mutational analysis of rhizobitoxine bioynthesis genes in *Bradyrhizobium elkanii*. Appl. Environ. Microbiol. 2001; 67: 4999–5009.
30. Yuhashi KI, Ichikawa N, Ezura H, Akao S, Minakawa Y, Nukui N, Yasuta T, Minamisawa K. Rhizobitoxine production by *Bradyrhizobium elkanii* enhances nodulation and competitiveness on *Macroptilium atropurpureum*. Appl. Environ. Microbiol. 2000; 66: 2658–2663.
31. Zhou W, Lin X. Effects of waterlogging at different growth stages on physiological characteristics and seed yield of winter rape (*Brassica napus* L.). Field Crops Res. 1995; 44: 103–110.
32. Zhou WJ. Oilseed rape cultivation. In: Ding YS (Editor). Cultivation of Crops. Shanghai Sci. and Tech. Press. Shanghai. 1994: 357–380.

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