

Effect and Role of Bio-Fertilizer in Agriculture

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Abstract – Bio-fertilizer are one of the best modern tools for Agriculture it is defined as preparations containing living cells or latent cells of efficient strains of microorganism that help crop plants, uptake of nutrients by their interactions in the rhizosphere which applied theory seed or soil. They accelerate certain microbial processes in the soil which argument the extract of availability of nutrients in a form easily assimilated by plants. It is a gift of our modern agricultural science. Bio-fertilizers are applied in the agricultural field as a replacement to our conventional fertilizers. Conventional fertilizers contain composite house hold wastes and green manner.

In order to study the combined effect of chemical phosphorus fertilizers those are not as effective a chemical fertilizers. So farmers often try to use chemical fertilizer in the field for crop development. But obviously the chemical fertilizers are not environment friendly. They are responsible for water, air and soil polluting and can spread cancer causing agents. Moreover, they may destroy the fertility of the soil in a long run. Bio-fertilizer, prevent pollution and to make this world healthy for everybody in a natural way. Bio-fertilizer contains microorganisms which promote the adequate supply of nutrient to the host plants and ensure their proper development of growth and regulation in their physiology. Living microorganisms are used in the preparation of bio-fertilizers only there organisms are used which have specific functions to enhance plant growth and reproduction. There are different types of microorganism which an used in the bio-fertilizers. Bio fertilizers being essential component of organic farming play vital role in maintaining soil fertility and sustainability.

Key Words: Azospirillum, Bio-Fertilizers, Crop Growth, Sustainability.

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INTRODUCTION

Use of bio-fertilizers is one of the important components, of integrated nutrient management, as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable and agriculture several microorganisms and their association with crop plant are being exploited in the production of bio-fertilizers organic farming has emerged as an important priority area globally in view of the growing demand for safe and healthy food and long their sustainability and concern on environmental pollution associated with indiscriminate use of agrochemical some of the important function are stimulate production of growth promoting substance like Vit. B Complex, Indole acetic acid (IAA) and Gibbrellic acids etc. though the use of chemical inputs in agriculture is inevitable to meet the growing demand for food in worlds. There are opportunities in selected crops and niche areas where organic production can be encouraged to tape the domestic export market. Bio-fertilizer are being essential component of organic farming are the preparation containing live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing of cellulolytic microorganisms used for application to seeds, soil on composting area with objective of

increasing number of such microorganism and accelerate those microbial processes which augment the availability nutrients that can be easily assimilated by plants. Bio-fertilizers play a very significant role in improving soil fertility by fixing atmospheric nitrogen, both in association with plant roots and without it, solubilize insoluble soil phosphates and produces plant growth substances in the soil. They are in fact being promoted to harvest naturally available biological system of nutrient mobilization. The role and importance of bio-fertilizers in sustainable crop production has been reviewed by several authors. But the progress in the field of BT production technology remained always below satisfaction in Asia because of various constraints.

NEED OF BIO-FERTILIZERS:

Indiscriminate use of synthetic fertilizers has led to the pollution and contamination of the soil, has polluted water basins, destroyed micro-organisms and friendly insects, making the crop more prone to disease and reduced soil fertility.

Demand is much higher than the availability. It is estimated that by 2020, to achieve the targeted

production of 321 million tons of food grains, the requirement of nutrient will be 28.8 million tons, while their availability will be only 21.6 million tones being a deficit of about 7.2 million tones.

Depleting feedstock/fossil fuels (energy crises) and increasing cost of fertilizers. This is becoming unaffordable by small and marginal farmers, depleting soil fertility due to widening gap between nutrient removal and supplies, growing concern about environmental hazards, increasing threat to sustainable agriculture. Besides above facts, the long term use of bio-fertilizers is economical, eco-friendly, more efficient, productive and accessible to marginal and small farmers over chemical fertilizers.

Different Types of Bio-Fertilizer:

Sl. No.	Groups	Examples
1.	Free-living	Azotobacter, Beijerinckia, Clostridium, Klebsiella, Anabaena, Nostoc,
2.	Symbiotic	Rhizobium, Frankia, anabaena Azollae
3.	Associative Symbiotic	Azospirillum
P Solubilizing Biofertilizers		
1.	Bacteria	Bacillus megaterium var. phosphaticum, Bacillus subtilis Bacillus circulans, Pseudomonas striata
2.	Fungi	Penicillium sp., Aspergillus awamori
P Mobilizing Bio-Fertilizers		
1.	Arbuscular mycorrhiza	Glomus sp., Gigaspor sp., Acaulospora sp., scutellospora sp. & sclerocystis sp.
2.	Ectomycorrhiza	Laccaria sp., pisolithus sp., Boletus sp., Amanita sp.
3.	Ericoid mycorrhizae	Pezizella ericae
4.	Orchid mycorrhiza	Rhizoctonia solani
Bio-Fertilizers for Micro nutrients		
1.	Silicate and Zin Solubilizers	Bacillus sp.
Plant Growth Promoting Rhizobacteria		
1.	Pseudomonas	Pseudomonas Fluorescens

Potential Characteristic Features of Some Bio-Fertilizers:

Nitrogen Fixers Rhizobium: belongs to family Rhizobiaceae, symbiotic in nature, fix nitrogen 500-100 kg/ ha in assoctatio with legumes only. It is useful for pulse legumes like chickpea, red-gram, paze, lentil, black gram, etc., oil-seed legumes like soybean and groundnut and forage legumes like berseem and Lucerne. Successful nodulation of leguminous crops by rhizobium largely depends on the availability of compatible strain for a particular legume. It colonizes the roots of specific legumes to form tumor like growths called root nodules, which acts as factories of ammonia production. Rhizobium has ability to fix atmospheric nitrogen in symbiotic association with legumes and certain non-legumes like **Parasponia**. Rhizobium population in the soil depends on the presence of legume crops in the field. In absence of legumes, the population decreases, Artificial seed inoculation is often needed to restore the population of effective strains of the Rhizobium near the rhizosphere to hasten N-fixation. Each legume requires a specific species of Rhizobium to form effective nodules.

Azospirillum: Belongs to family **Spirilaceae**, heterotrophic and associative in nature. In addition to their nitrogen fixing ability of about 20-40 kg/ha, they also produce growth regulating substances.

Although there are many species under this genus like, *A. amazonense*, *A. halopaeferens*, *A. brasilense*, but, worldwide distribution and benefits of inoculation have been provided mainly with the *A. lipoferum* and *A. brasilense*. The *Azospirillum* from associative symbiosis with many plants particularly with those having the C4-dicarboxylic path way of photosynthesis (Hatch and Slack pathway), because they grow and fix nitrogen on salts of organic acids such as malic, aspartic acid. Thus it is mainly recommended for maize, sugarcane, sorghum, pearl millet etc. the *Azotobacter* colonizing the roots not only remains on the root surface but also a sizable proportion of them penetrates into the root tissues and lives in harmony with the plants. They do not, however, produce any visible nodules or out growth on root tissue.

Azotobacter: Belongs to family **Azotobacteriaceae**, aerobic, free living, and heterotrophic in nature. *Azotobacter* are present in neutral or alkaline soils and *A. Chroococcum* is the most commonly occurring species in arable soils. *A. vinelandii*, *A. beijerinckii*, *A. insignis* and *A. macrocytogenes* are other reported species. The number of *Azotobacter* rarely exceeds of 10⁴ to 10⁵g-l of soil due to lack of organic matter and presence of antagonistic microorganisms in soil. The bacterium produces anti-fungal antibiotics which inhibits the growth of several pathogenic fungi in the root region thereby preventing seedling mortality to a certain extent. The population of *Azotobacter* is generally low in the rhizosphere of the crop plants and in uncultivated soil. The occurrence of this organism has been reported from the rhizosphere of a number of crop plants such as rice, maize, sugarcane, bajra, vegetables and plantation crops.

Blue Green Algae (Cyanobacteria) and Azolla: These belongs to eight different families, phototrophic in nature and produce Auxin, Indole acetic acid and Gibberlic acid, fix-20-30 kg N/ha is submerged rice fields as they are abundant in paddy, so also referred as 'paddy organisms'. N is the key input required in large quantities for low land rice production. Soil N and BNF by associated organisms are major sources of N for low land rice. The 50-60% N requirement is met through the combination of mineralization of soil organic N and BNF by free living and rice plant associated bacteria. To achieve food security through sustainable agriculture, the requirement for fixed nitrogen must be increasingly met by BNF rather than by industrial nitrogen fixation.

BGA forms symbiotic association capable of fixing nitrogen with fungi, liverworts, ferns and flowering plants, but the most common symbiotic association has been found between a free floating aquatic fern, the *Azolla* and *Anabaena azollae* (BGA). *Azolla* contains 4-5% N on dry basis and 0.2-0.4% on wet basis and can be the potential source of

organic manure and nitrogen in rice production. The important factor in using Azolla as biofertilizer for rice crop is its quick decomposition in the soil and efficient availability of its nitrogen to rice plants. Besides N-fixation, these biofertilizers or biomanures also contribute significant amounts of P, K, S, Zn, Fe, Mb and other micronutrient. The fern forms a green mat over water with a branched stem, deeply bilobed leaves and roots. The dorsal fleshy lobe of the leaf contains the algal symbiont within their central cavity. Azolla can be applied as green manure by incorporating in the fields prior to rice planting. The most common species occurring in India is *A. pinnata* and same can be propagated on commercial scale by vegetative means. It may yield on average about 1.5 kg per square meter in a week. India has recently introduced some species of Azolla for their large biomass production, which are *A. caroliniana*, *A. microphylla*, *A. filiculoides* and *A. Mexicana*.

Phosphate Solubilizers: Several reports have examined the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate. Among the bacterial genera with this capacity are *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aerobacter*, *Flavobacterium* and *Erwinia*. There are considerable populations of phosphatesolubilizing bacteria in soil and in plant rhizospheres. These include both aerobic and anaerobic strains, with a prevalence of aerobic strains in submerged soils. A considerably higher concentration of phosphate solubilizing bacteria is commonly found in the rhizosphere in comparison with non rhizosphere soil. The soil bacteria belonging to the genera *Pseudomonas* and *Bacillus* and Fungi are more common.

Phosphate Absorbers (Mycorrhiza): The term Mycorrhiza denotes "fungus roots". It is a symbiotic association between host plants and certain group of fungi at the root system, in which the fungal partner is benefited by obtaining its carbon requirements from the photosynthates of the host and the host in turn is benefited by obtaining the much needed nutrients, especially phosphorus, calcium, copper, zinc, etc, which are otherwise inaccessible to it, with help of the fine absorbing hyphae of the fungus. These fungi are associated with majority of agricultural crops, except with those crops / plants belonging to families of *Chenopodiaceae*, *Amaranthaceae*, *Caryophyllaceae*, *Polygonaceae*, *Brassicaceae*, *Commelinaceae*, *Juncaceae* and *Cyperaceae*.

Zinc Solubilizers: The nitrogen fixers like *Rhizobium*, *Azospirillum*, *Azotobacter*; BGA and Phosphate solubilizing bacteria like *B. magisterium*, *Pseudomonas striata*, and phosphate mobilizing Mycorrhiza have been widely accepted as bio-fertilizers. However these supply only major nutrients but a host of microorganism that can transform micronutrients are there in soil that can be used as

bio-fertilizers to supply micronutrients like zinc, iron, copper etc., The zinc can be solubilized by microorganisms viz., The results have shown that a *Bacillus* sp. (Zp solubilizing bacteria) can be used as bio-fertilizer for zinc or in soils where native zinc is higher or in conjunction zinc carbonate ($ZnCO_3$) and zinc sulphide (ZnS) instead of costly zinc sulphate.

Potential Role of Bio-Fertilizers in Agriculture:

The incorporation of bio-fertilizers (N-fixers) plays major role in improving soil fertility, yield attributing characters and thereby final yield has been reported by many workers. In addition, their application in soil improves soil biota and minimizes the sole use of chemical fertilizers.

Under temperate conditions, inoculation of *Rhizobium* improved number of pods plant⁻¹, number of seed pod⁻¹ and 1000-seed weight (g) and thereby yield over the control. The number of pods plant⁻¹, number of seed pod⁻¹ and 1000-seed weight. In rice under low land conditions, the application of BGA+ *Azospirillum* proved significantly beneficial in improving LAI and all yield attributing aspects.

It is an established fact that the efficiency of phosphate fertilizers is very low (15-20%) due to its fixation in acidic and alkaline soils and unfortunately both soil types are predominating in India accounting more than 34% acidity affected and more than seven million hectares of productive land salinity/alkaline affected. Therefore, the inoculations with PSB and other useful microbial inoculants in these soils become mandatory to restore and maintain the effective microbial populations for solubilization of chemically fixed phosphorus and availability of other macro and micronutrients to harvest good sustainable yield of various crops.

MATERIALS AND METHODS:

Combined effects of phosphorus fertilizer, phosphate-solubilising bacteria and mycorrhizal fungus were determined on reducing drought stress damages of grain corn (KSC704 commercial hybrid) under field conditions in experimental farm. The experiment was planted in 2015 as a randomized complete block design with split-plot arrangement and three replications. The soil texture was clay loam and the result of soil analysis presented in Table 1.

Treatment consisted of three levels of drought stress: without stress (irrigation after 50 mm evaporation from pan class A), low drought stress (irrigation after 100 mm evaporation from pan class A) and severe drought stress (irrigation after 150 mm evaporation from pan class A). In sub-plots five compound fertilizer such as: (b1) phosphate-solubilising bacteria with Mycorrhiza fungus, (b2)

phosphate-solubilising bacteria and Mycorrhiza fungi with 50% super phosphate triple, (b3) phosphate-solubilising bacteria with 50% super phosphate triple, (b4) Mycorrhiza fungi with 50% super phosphate triple and (b5) phosphate chemical fertilizer (100% super phosphate triple) were used.

Phosphate solubilising microorganisms used in this experiment were included Mycorrhiza fungi (*Glomus mosseae*) (with 65-70% colonization rate). Also biofertilizer phosphate-solubilising bacteria (biophosphor ®) was included *Bacillus* and *Pseudomonas* with CFU=107.

Plants were grown in five-row plots with 5 m length and 0.75 cm spacing between rows. The plant density was 66000 plant/ha. Fertilizer was used based on soil test. Irrigation was performed on class A evaporation pan for each treatment. Data was recorded on 10 competitive plants of each plot and grain yield (kg ha⁻¹) was calculated for the entire plot. Each plot was harvested at maturity for yield and yield components and leaf area index and dry matter were measured each 15 days to calculate Crop Growth Rate (CGR), Net Assimilation Rate (NAR) and Leaf Area Index (LAI) according to below equations:

$$(1) \quad LAI = LA / SA$$

$$(2) \quad CGR = (W_2 - W_1) / SA(t_2 - t_1) \text{ g.m}^{-2}.\text{day}^{-1}$$

$$(3) \quad NAR = (W_2 - W_1) / (t_2 - t_1) * (\ln LA_2 - \ln LA_1) / (LA_2 - LA_1) \text{ mg.mm}^{-2}.\text{day}^{-1}$$

In above abbreviations: LA = Leaf Area, SA = Ground area that occupied a plant. W = Dry matter, t = Day after planting.

Data was subjected to Analysis Of Variance (ANOVA) and the treatment means was compared using Duncan's multiple range test (alpha = 5%). The analysis was done by MSTATC and SAS (Ver. 9.1) software. Microsoft office Excel was used for figures drawing and indices calculation.

RESULTS:

Analysis of variance showed that there are significant differences among most traits (Table 2). A significant effect of stress levels treatments on grain yield of maize. The highest grain yield of 12.08 ton/ ha was obtained for the normal irrigation treatment and lowest (3.55 ton/ha) for the severe Drought stress treatment (Table 3). The results showed that stress treatment significantly reduced (P < 0.05) grain yield, percent colonization and harvest index. There were no significant differences in stress levels for row number in ear but there was a significant difference in fertilizer compounds. The other researcher showed that drought stress declined seed yield and

its components (Reca et al., 2001; Seghatoleslami et al., 2008).

All the assessed traits in b2 compound inoculate treatment were of higher values than other treats under drought stress condition. Furthermore, the investigated traits of b5 treat under severe drought stress were significantly less pronounced than normal irrigation and low stressed conditions. This finding was in agreement

Table 2. Analysis of variance of measured traits of corn under different fertilizer treatments and drought stress conditions.

S.O.V	df	Row No./ Ear	Kernel No./Row	300 Kernel weight	Ear length	Total Yield	Percent colonization	Harvest index
Replication	2	6.22ns	214.60ns	247.83ns	27.75ns	10.0**	14.04*	61.79*
Stress	2	18.52ns	2473.51**	2679.04*	193.86*	296.42**	638.77**	2329.07**
Main error	4	4.25	79.45	157.70	15.02	3.27	1.02	21.81
Fertilizer	4	8.18**	158.2**	164.58**	14.11**	20.49**	2842.30**	199.00**
Stress*Fertilizer	8	0.55ns	15.76ns	67.12ns	1.52ns	1.00ns	44.69**	10.39ns
Sub error	24	1.23	23.20	31.11	1.99	0.82	3.60	16.51
Mean		13.72	40.17	66.93	18.47	8.53	40.42	42.00

*, **, ns : significant at 5%, 1% level and not significant, respectively.

Table 3: Means comparison of measured traits of corn under different fertilizer treatments and drought stress conditions using Duncan's multiple range test.

	Row No./ Ear	Kernel No./ Row	300 Kernel Weight (gr)	Ear Length (cm)	Total Yield (ton/ha)	Percent colonization (%)	Harvest index (%)
Irrigation							
Normal irrigation	14.456a	49.22a	78.004a	21.222a	12.087a	45.47a	52.42a
Low drought stress	14.26a	45.82a	70.712a	19.807a	9.9712b	43.03b	45.37b
Severe drought stress	12.444a	25.479b	52.089b	14.41b	3.5507c	32.75c	28.20c
Fertilizer							
b1	14.445a	42.95ab	69.858ab	18.9263ab	9.3667b	57.93a	45.68a
b2	14.6303a	45.25a	72.44a	20.2215a	10.352a	23.39b	46.72a
b3	13.746a	39.39b	65.239bc	18.121bc	7.815c	27.07d	38.08b
b4	13.574a	39.013b	65.644bc	18.3509b	8.7463b	47.07c	43.44a
b5	12.21b	34.27c	61.487c	16.7778c	6.4014d	16.63e	36.07b

Means with same phrases in each columns not significant at 5% probability with the results of Ehteshami et al. (2009).

A combination of Mycorrhiza fungi and phosphatesolubilising bacteria had effects on these traits although, there were no significant differences among b2 and b5 for traits except for grain yield and percent colonization. Our results concur partly with observations made by Ehteshami et al. (2012) who reported that Mycorrhiza fungies and phosphate-solubilising bacteria increased traits.

According to this experiment result, under drought stress condition, seed inoculums with b2 treatment significantly affected the reduction of plant damages and therefore increased the total yield. Results of this experiment showed that phosphate-solubilizing microorganisms can positively interact with promoting plant growth as well as with phosphorus uptake in maize plant, leading to plant tolerance improved under drought stress conditions.

Leaf area index with the use of b1 was 3.8, in addition to normal irrigation maximum LAI was obtained among other levels (Figure 1). Application chemical fertilizer treatment alone in different levels

of irrigation observed that LAI in this treatment was decreased (Figure 2). This result showed that biological fertilizer to obtain relative resistance opposes the drought stress in corn. The lowest LAI at flowering stage was in b5 (2.85) and drought stress treatment (2.49).

The other researcher showed that the highest LAI was in 8 days period of irrigation (5.1) and drought stress in this treatment decreased 11% in LAI (Jafari et al., 2010).

Crop growth rate in b2 during the experiment was increased compared to other treatment (Figure 3). Probably in this experiment the crop growth rate is related to leaf area index, for the reason that crop growth changing rate is depended on two parameters: Namely leaf area index and net assimilation rate. This finding was in agreement with the results of Brogeham (2000). At flowering stage (75-90 day after planting), Severe drought stress condition had decreased CGR index compared to other irrigation treatments (Figure 4). Net assimilation rate with the use of b2 was increased compared to other treatments (Figure 5). Other fertilizer compounds were not different from each other (Figure 5). The normal irrigation and low drought stress had overlapped on each other (Figure 6).

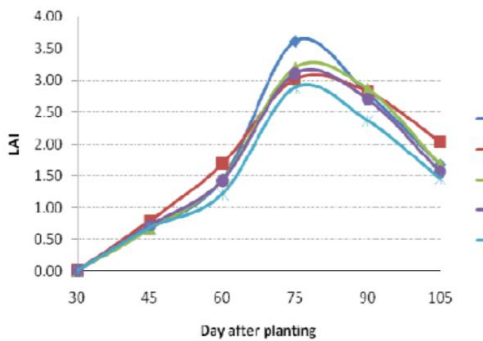


Figure 1. Leaf area index in five fertilizer treatment in during period of growth.

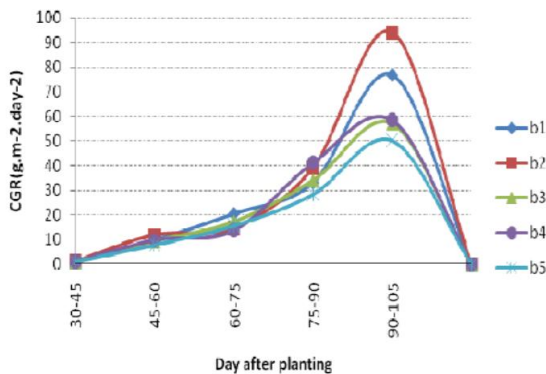


Figure 3. Crop growth rate in five fertilizer treatment in during period of growth.

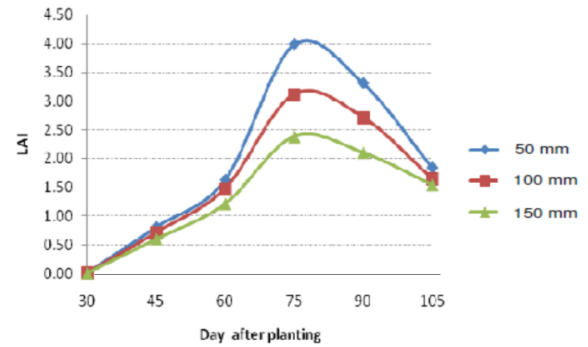


Figure 2. leaf area index in different levels irrigation in during period of growth.

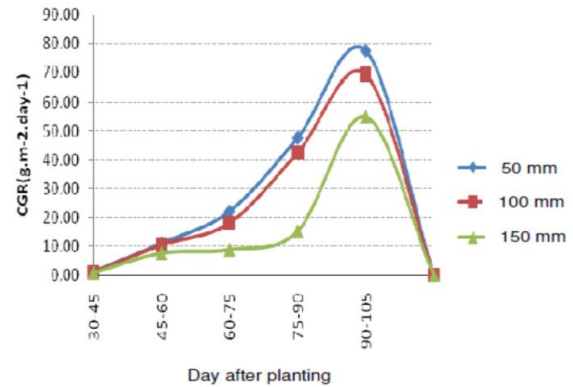


Figure 4. Crop growth rate in different levels irrigation in during period of growth.

In biological fertilizer compounds the highest NAR was 0.055 (mg.mm⁻².day⁻¹). Result of this experiment showed that chemical fertilizer used under drought stress condition can decrease NAR index more than biological fertilizer. Balak (1993) showed that NAR would decrease with the increase of LAI. This trend of decrease will continue from the beginning till the end of growth season.

CONCLUSION:

Results of this study showed that, drought stress caused decreases of yield and its component and measured indices such as LAI, CGR and NAR. Drought stress is a major abiotic constraint responsible for heavy production losses (Khan et al., 2007).

Application of biological fertilizer (b2) has given the highest grain yield in this study. El-Karmany (2001) showed that integrated chemical and biological fertilizer obtained highest kernel number per year compared to sole application of them (4). Also our study showed that chemical fertilizer combined with biological fertilizer was beneficial to the environment because with decreasing the use of chemical fertilizer and use of organic inputs we can side with sustainable agriculture, and increase the efficiency of water.

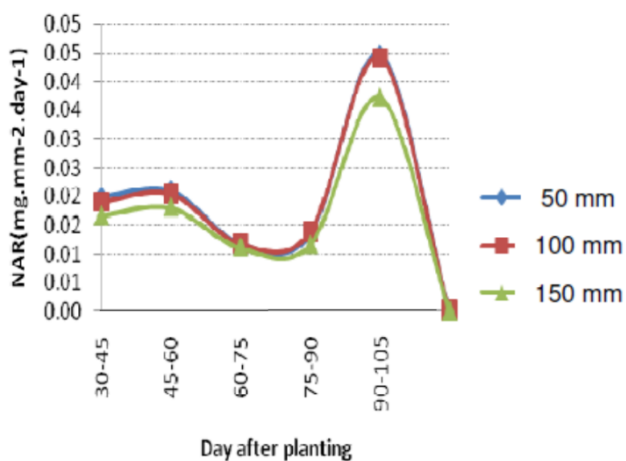
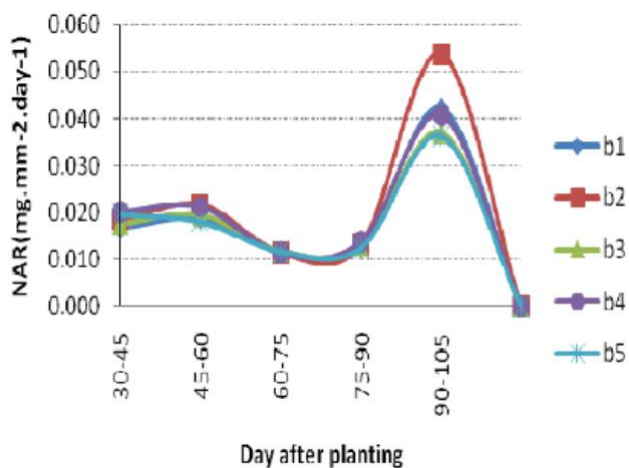


Figure 6. Net assimilation rate in different levels irrigation in during period of growth.

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