RESEARCH PAPER

Asian Journal of Bio Science Vol. 6 Issue 2 (Oct., 2011) : 201-206

Effect of *Azotobacter* spp. inoculum on growth of wheat variety trimbak

R. H. AUTADE, A.S. PATIL, D.K. JADHAV, Y. SUTAR AND A M. BHOSALE

Department of Plant Biotech, College of Agricultural Biotechnology, Loni, AHMEDNAGAR (M.S.) INDIA

E-mail: rishiz.utade@gmail.com

(**Received:** May, 2011; **Revised:** Jul., 2011; **Accepted :** Sep., 2011)

Biofertilizer has been acknowledged as a substitute to chemical fertilizer to increase soil fertility and crop production in sustainable farming. Most of the farmers assume that chemical fertilizer gives more yield than the biofertilizer, ignoring environmental and long term losses. This study is done to test the efficiency and efficacy of the biofertilizer in opposition to the chemical fertilizer and in additions to this comparison also done among the four species of *Azotobacter* for their aid in increase in yield and biomass of wheat crop. The field experiment was conducted during *Rabi* 2006-07 season using randomized Block Design and Trimbak variety were used for sowing in 21 plots. About nine parameters of wheat crop were selected and intermittently readings were taken at 30, 60, 90 days of interval to observe alteration, due to use of biofertilizer, chemical fertilizer and some plots were kept as a control for comparison. ANOVA were used to test the significance in treatments.

Key words : Biofertilizer, *Azotobacter*, Wheat, Chlorophyll

Autade, R.H., Patil, A.S., Jadhav, D.K., Sutar Y. and Bhosale, A.M. (2011). Effect of *Azotobacter* spp. inoculum on growth of wheat variety trimbak. *Asian J. Bio. Sci.*, **6** (2) : 201-206.

INTRODUCTION

Wheat is a largest used food crop in India. Mainly cultivation is done in Maharashtra, Punjab, Andhra Pradesh, Gujarat etc; Chemical fertilizers are mostly used for production of wheat, chemical fertilizers are harmful and cause soil pollution. This can affect product yield. Biomagnifications occurs which leads to disturbance in food chain (Merrington G, 2001). The cost of chemical fertilizer is increasing day by day and also not economical due to leaching and volatilization losses. Various kinds of biofertilizer can be used for this purpose. Nitrogen is main component for plant growth. Therefore, biofertilizer have achieved a special significance in modern agriculture. Biofertilizer are the good available choice in our hand to supply essential nutrients to the crop in biological ways without deleterious effect. Nitrogen fixing bacteria fixes nitrogen. Thus they are used as biofertilizer for many crops e.g. brinjal, cotton, wheat and groundnut (Tulsa Ram, 2005). Nitrogen fixing bacteria fixes nitrogen [Non-symbiotically] *Azotobacter*, *Clostridium* and [symbiotically] *Rhizobium*.

The genus *Azotobacter* comprises large, Gram-ve obligate aerobic rods, capable of fixing nitrogen non-symbiotically. It is roughly estimated that *Azotobacter* spp. can fix 10 to 15 kg. N_2/ha/annum (Badgire D.R, 1976). *Azotobacter chroococcum* is used as a bioinoculant known in benefit a wide variety of crops due to secretion of growth promoting substances, Vitamin B, antifungal, metabolites and phosphate volatilization which increase seed germination and plants stand and also improve the initial vigour of inoculated plants (Subba Rao, 1993). *Azotobacter* fixes atmospheric nitrogen in the rhizosphere region *i.e.* soil around the seedling or trees. Biofertilizer applied to seed or seedlings, bacteria remain around seeds or seedlings and use organic carbon for their metabolism. When seeds are germinated or seedlings set in soil they leave or exude root exudates which become food for these bacteria. They grow on these substances which include sugars, organic acids, and amino acids and fix atmospheric nitrogen most efficiently. Nitrogen so fixed by these bacteria becomes available to plants after dead and degradation of bacterial cells.

RESEARCH METHODOLOGY

Field experiment study was conducted at Biotechnology Department, Padmashri Vikhe Patil College, Loni. The soil of selected plot was medium black. The source of *Azotobacter* spp. was obtained from Botany department of P. V. P. College, Loni. For the experiment following *Azotobacter* spp. were used.

– *A. vinelandii* [CMI (P)] T_1
– *A. beijerinckii* [BVA(P)] T_2
– *A. macrocytogenes* [CMI(M)] T_3
– *Azospirillum spp.* [BVA(M)] T_4

Design of plots:

The field experiment was conducted during *Rabi* 2006-07 season using Randomized Block Design (V. Zecevic 2010). Each treatment is given as below:-

21 Plots were selected. The experimental area was marked with its definite boundaries and beds were prepared.

Plot size: - 5 m × 4 m.

Layout of plot

Plot No.34 Control [T_6]	Plot No.35 Recommended [T_5]	Plot No.36 CMI(P) [T_1]
Plot No.31 CMI (P) [T_1]	Plot No.32 BVA(P) [T_2]	Plot No.33 CMI(M) [T_3]
Plot No.28 BVA(P) [T_2]	Plot No.29 Control [T_6]	Plot No.30 BVA(M) [T_4]
Plot No.25 BVA(M) [T_4]	Plot No.26 CMI(M) [T_3]	Plot No.27 Control [T_6]
Plot No.22 Control [T_6]	Plot No.23 Recommended [T_5]	Plot No.24 CMI(P) [T_1]
Plot No.19 CMI(M) [T_3]	Plot No.20 BVA(M) [T_4]	Plot No.21 Recommended [T_5]
Plot No.16 Recommended [T_5]	Plot No.17 CMI(P) [T_1]	Plot No.18 BVA(P) [T_2]

Seed inoculation with *Azotobacter* inoculums

Seeds of wheat variety Trimbak obtained from market were used for this experiment. The total seed requirement was calculated at rate of 125 kg. /ha. In each plot 30 g of wheat seeds were sown in 10 rows.

Azotobacter treatments were given to the wheat seeds by dressing treatment in which 10 ml of *Azotobacter* culture was taken in which 20 g of sugar was added. Out of which 2 ml was pipette out in flask and wheat seeds were poured for 20 seconds. All these treatments were carried out in Laminar Air flow. The drying of seeds was done in shade, after this seeds were sown (Jones J.P, 1978).

Fertilizers:

Recommended plots were provided with fertilizers including P_2O_5 and K_2O. Additionally urea was also used (Vipin Kumar, 2010).

Sowing:

The plots were marked with marker maintaining 22.5 cm spacing between rows. The seeds were then sown by hand dibbling in lines according to each treatment. The sowing was done on 17th Nov.2006.

Irrigation and tillage operations:

The plots were lightly irrigated immediately after sowing and thereafter, irrigations were given as and when required. In all 6 irrigations were given. Three weeding were done at an interval of three weeks. The sprays each of rogar and diethane –Z- 78 were given at an interval of 15 days. First spray was done before flowering and second spray was done after flowering to control aphids and wheat rust, respectively (Fathi A Mubeen, 2006).

Observation were taken after 30, 60, 90 days of wheat growth. These were tabulated in a form of observation table (Table 1, 2, 3). From each plot 3 replicates were selected randomly to minimize the error in readings of the following parameters:

Parameters studied after 30 days of wheat growth are:

Height of plant, no. of leaves, no. of tillers, fresh weight, dry weight and chlorophyll contents.

Parameters studied after 60 days of wheat growth are:

Height of plant, no. of awns, length of awns, no. of leaves, no. of tillers, fresh weight, dry weight and chlorophyll contents

Parameters studied after 90 days are:

Height of plant, no. of awns, length of awns, no. of leaves, no. of tillers, dry weight and no.of grains

Chlorophyll estimation:

Wheat leaves were washed with distilled water and dried with filter paper. 200 mg of leaves were cut with help of sterile blade. Leaves were crushed in acetone with the help of pestle and mortar. Extract was passed through the filter paper, into a clean test tube. Final volume of filtrate made 10ml with acetone (Jinheng Zhang, 2009).

O.D. measured using colorimeter at 620 and 660 wavelength.

Formulae for chlorophyll estimation:-
For chlorophyll b:

$$x = \frac{[12.7 \text{ x } A660 - 2.69xA620]x10x100}{1000x \text{ weight of plant material}}$$

For chlorophyll a:

$$Y = \frac{[22.9xA620 - 4.68xA660]x10x100}{1000 \text{ x weight of plant material}}$$

$$z = \frac{[20.2xA660+8.02xA620]x10x100}{1000x \text{ weight of plant material}}$$

Thus, by using above formulae chlorophyll estimation was done.

RESULTS AND ANALYSIS

The results are summarized below according to objectives of the study:

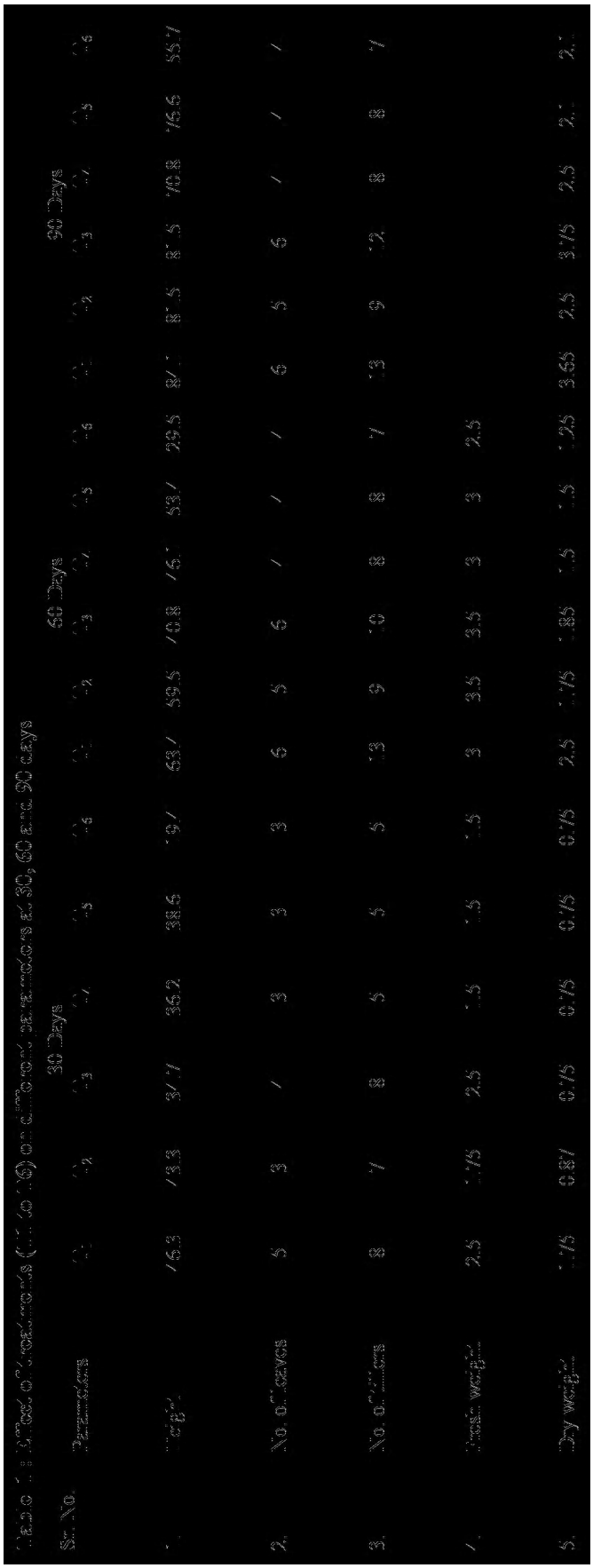
Field experiment:

At 30, 60 and 90 days average plants height differs significantly from T_1 to T_6. Average maximum height at 30, 60 and 90 days was (46.3cm), (63.4cm) and (84.1cm), respectively, in T_1 treated plots (Table 1). Readings of observations were plotted in the histogram with height on Y-axis and treatments on X-axis. T_1 treated plot grew with more height as compared to other treatment. Same observations followed in plots with minute deviation due to environmental consequence. (Fig. 1a)

Data represented in (Table 1) revealed that at 30 days maximum number of leaves (5) and tillers (8) was recorded in T_1 treated plots as compared to other treatments, when results were recorded at 60 and 90 days. T_1 treated plots have shown superior performance when fresh and dry weights were measured. T_2 treated plots have shown some noteworthy, but slightly low performance than T_1 treated plots. (Fig.1b)

Similarly, (Fig. 2) when chlorophyll content of leaves were checked for all plots, among six treatments, T_1 treated plots shown significant increase in chlorophyll content, at 60 days it was recorded (b-0.0941,a-0.0695 and z-0.2109) as compared to other T_2 to T_5 treatments when readings were recorded after 30 and 60 days. (Table 2)

The (Fig. 3) depicts the effect of six treatments on remaining accessible parameter at 60 and 90 days. Reading for parameter no. 7 and 8 were taken after 60 and 90 days. For parameter no.9 readings were taken after 90 days. Number of grains per spike at 90 days



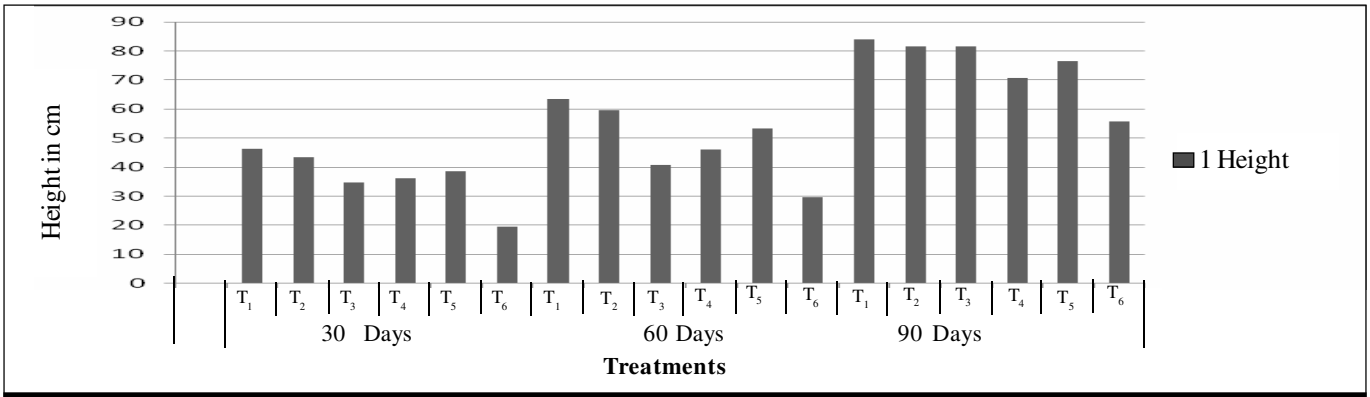


Fig. 1a : Effect of six treatments on height of wheat crop at 30, 60 and 90 days is plotted

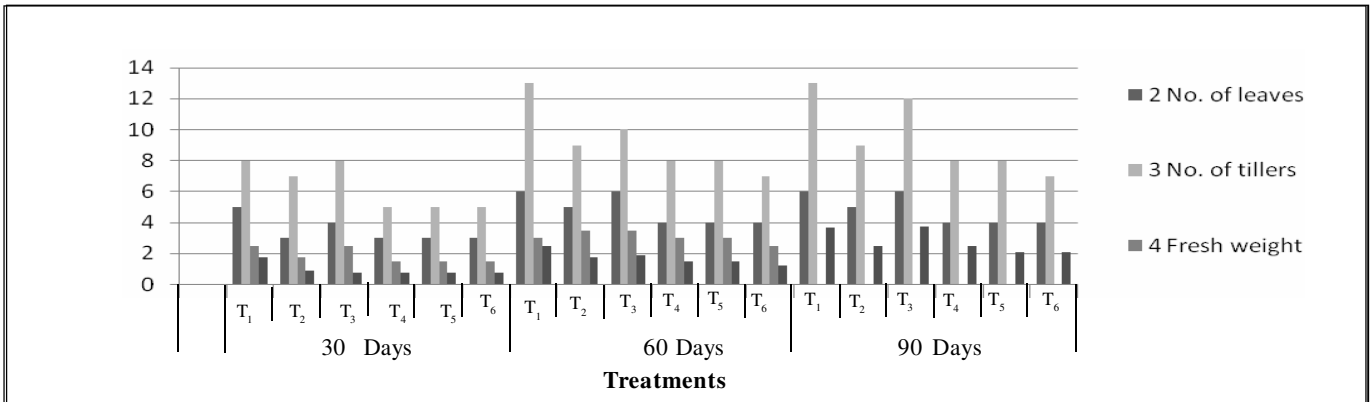


Fig. 1b: Effect of six treatments on parameter no.2, 3, 4 and 5 at 30, 60 and 90 days are plotted

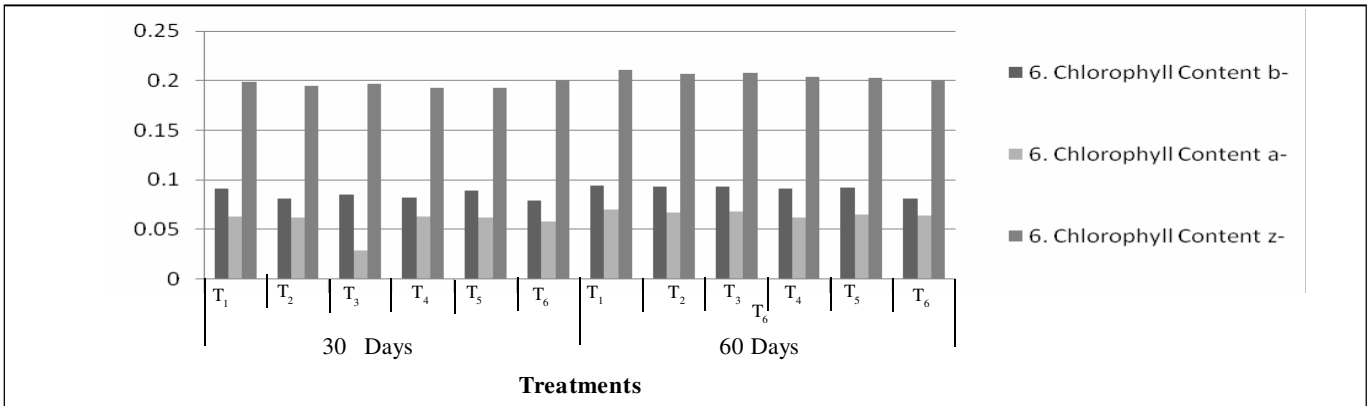


Fig. 2: Effect of six treatments on chlorophyll content at 30, 60 and 90 days, is plotted

Table 2 : Effect of treatments (T1 to T6) on chlorophyll content at 30 and 60 days

Sr. No.	Parameter		30 Days T1	T2	T3	T4	T5	T6	60 Days T1	T2	T3	T4	T5	T6
6.	Chloro-phyll Content	b-	0.091	0.081	0.085	0.082	0.089	0.079	0.0941	0.0932	0.0932	0.0909	0.0916	0.0811
		a-	0.063	0.0622	0.029	0.0625	0.0616	0.058	0.0695	0.0668	0.0677	0.0622	0.0653	0.064
		z-	0.199	0.1951	0.197	0.1925	0.1928	0.1994	0.2109	0.2067	0.2081	0.204	0.2028	0.1994

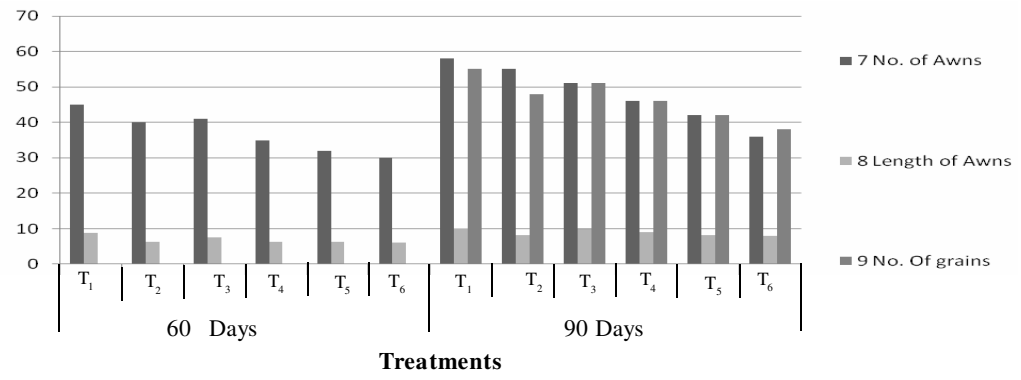


Fig. 3: Effect of six treatments on parameter no.7, 8 and 9 at 30, 60 and 90 days are plotted

Table 3 : Effect of treatments (T1 to T6) on different parameters at 60 and 90 days

Sr. No.	Parameters	60 Days						90 Days					
		T_1	T_2	T_3	T_4	T_5	T_6	T_1	T_2	T_3	T_4	T_5	T_6
7.	No. of awns	45	40	41	35	32	30	58	55	51	46	42	36
8.	Length of awns	8.9	6.25	7.5	6.25	6.2	6	10	8.3	9.8	9.1	8.3	8
9.	No. of grains	-	-	-	-	-	-	55	48	51	46	42	38

revealed that average numbers of grains differed significantly from T_1 to T_6 and yet again T_1 treated plots shown fine outcome (55 grains), superiority in quality and quality as compared to *Azotobacter* treated plots outcome (Table 3).

So, from all the tabulated observations, it confirms that there is considerable improvement observed in all different parameters, when the four species of *Azotobacter* treated seeds were sown in 13 plots as compared to control four plots and recommended four plots, in which performances of different parameters after regular interval of 30, 60 and 90 days were found slightly less efficient.

Considering the all histogram statistics and layout of plots, it can be concluded that treatments of *Azotobacter* spp. given to plots 17, 18, 19, 20, 24, 25, 26, 28,30,31,32,33 and 36 shown exceedingly competent growth.

While recommended plot No.16, 23, 21, 35 *i.e.* Plots provided with chemical fertilizers have shown moderately efficient growth and control plot No.22, 27, 29, and 34 have shown less efficient growth. Among the treatments T_1, T_2, T_3, T_4, T_5 and T_6, the *A. vinelandii* CMI (P) *i.e.* T_1 have shown extremely efficient growth than, T_2, T_3, T_4, T_5, and T_6.

The data were analyzed statistically by ANOVA techniques; test at per cent probability level was applied to compare the differences among the treatment means (*Ali et al.,* 2003).

ANOVA for testing significance in data:

Null Hypothesis

H_0: There is no significant difference in effect of six treatments on wheat growth.

H_1: There is significant difference in effect of six treatments on wheat growth.

Table 4 : ANOVA result of all ten parameters

Sr.No.	Parameter	$F_{calculated}$	*P-value*	$F_{critical}$*
1.	Height of plant	16.60791	0.000157	3.68232
2.	No. of Leaves	4.050633	0.039212	3.68232
3.	No. of tillers	4.280105	0.033833	3.68232
4.	Fresh Weight	22.72973	0.00076	4.964603
5.	Dry weight	16.74942	0.000151	3.68232
6.	Chl-b	5.093676	0.047617	4.964603
7.	Chl-a	19.8789	0.001219	4.964603
8.	Chl-z	26.31043	0.000445	4.964603
9.	No. of Awns	6.919424	0.025141	4.964603
10.	Length of Awns	12.71374	0.005133	4.964603

* and ** indicate significance of values at P=0.05 and 0.01, respectively

As shown in (Table 4), for all parameters, calculated value *i.e.* $F_{calculated}$ is greater than the table value *i.e.*

$F_{critical}$*. Also, the P value is less than Alfa value *i.e.* 0.05. Hence, we reject the H_0 (Null Hypothesis) and accept the H_1 hypothesis. So, there is significant difference in effect of six treatments on wheat growth.

So, for 1st parameter we can conclude that, there is high significant difference (f= 16.6079*, P=0.0001) between the height of wheat plant, when height were recorded at 30, 60 and 90 days in T_1 to T_6 treated plots.

Acknowledgement:

The authors are grateful to Dr. A. M. Bhosale, HOD of Biotechnology, Padmashri Vitthalrao Vikhe Patil college, Loni, India for identifying and providing the species of Azotobacter. The authors are also thankful to Dr. B. J. Apparao, Principal, Jijamata Senior College, Bhenda for providing valuable inputs during analyzing the data. We thank the Padmashri Vitthalrao Vikhe Patil College, Loni, India for providing facilities to conduct the study.

LITERATURE CITED

Abbas Akbari, Gh., Arab,Seyyed Mehdi, Alikhani, H.A., Allahdadi, I. and Arzanesh, M.H. (2007). Isolation and selection of indigenous *Azospirillum* spp. and the IAA of superior strains effects on wheat roots. *World J. Agric. Sci.* **3** (4): 523-529.

Ali Liaqat, Q. Mohy-ud-Din and Ali, M. (2003). Effect of different doses of nitrogen fertilizer on the yield of wheat. *Internet.J. Agric.& Biol.,* **5**(4):438-439.

Asad Rokhzadi and Vafa Toashih (2011). Nutrient uptake and yield of chickpea (Cicer arietinum L.) inoculated with plant growth promoting rhizobacteria. *AJCS* **5**(1):44-48.

Bahrani, A., Pourreza and Hagh Joo, J. M. (2010). Response of winter wheat to co-inoculation with *Azotobacter* and Arbescular Mycorrhizal Fungi under different sources of Nitrogen Fertilizer. *American- Eurasian J. Agric & Environ. Sci.,* **9** (4): 376-384.

Badgire, D.R. and Bindu K.J. (1976). Effect of *Azotobacter* seed inoculant on wheat (*Triticum vulgare*) *Madras Agric J.* **63** (11-12) 603-605.

Brown E.M; Burligham, S.K. and Jackson R.M. (1964). Studies on *Azotobacter* spp. in soil–Effect of artificial inoculation on crop yield. *Plant Soil* **20**, 194-214.

Dubey R.C. and D.K. Maheshwari. (2005). *Practical Microbiology* S. Chand and Co. Ltd., Ram Nagar, New Delhi.

Fathi A. Mubeen, Aama aslam, Munir, A. Sheikh, Tahira Iqbal, Sohail hammed, Kausar A., Malik and Fauzia Y. hafeez (2006). Response of wheat yield under combined use of fungicides and biofertilizer Internet. *J. Agric. Biol.* **8** (5):580–582.

Jones, J.P and Collins, F.C. (1978). Evaluation of *Azotobacter* seed inoculants for nitrogen fixation wheat and oats. *Arkansas farm Res.* **28** (2) : 102-106.

Jinheng Zhang1, Chao Han1 and Zhiheng Liu.(2009). Absorption spectrum estimating rice chlorophyllm concentration: Preliminary investigations. *J. Plant Breeding &Crop Sci.* Vol. **1**(5). pp. 223-229.

Mehrotra *et al;* (1971). Effect of *Azotobacter* inoculation on crop yields. *J. Ind. Soc. Soil, Sci.* **19**: 243-248.

Merrington G., Miller, D., McLaughlin, M.J., Keller, M.A. (2001). Trophic barriers to fertilizer Cd bioaccumulation through the food chain: a case study using a plant—insect predator pathway. *Arch Environ Contam Toxicol,* **41**(2):151-6.

Nasser, Kh., El-Gizawy, B. (2009). Effect of planting date and fertilizer application on yield of wheat under no till system. *World J.Agric. Sci.* **5** (6): 777-783.

Subba Rao, N.S. Venkatraman, G.S. and Kannaiyan. S. (1993). *Azotobacter* in –Bioligical Nitrogen fixaion. ICAR Pub. New Delhi PP. 39-42.

Tejera, N., Lluch,C., Martýnez-Toledo, M.V. and J. Gonzalez-L opez (2005). Isolation and characterization of *Azotobacter* and *Azospirillum* strains from the sugarcane rhizosphere. *Plant & Soil* **270**: 223–232.

Tulsa, Ram, Yadav, S. K. and Sheoran, R. S. (2005). Growth analysis of wheat (*Triticum aestivum L.*) under varying fertility levels and *Azotobacter* strains. *Indian J. Agric. Res.* **39** (4): 295-298.

Umar, Iqbal, Kumar Wali,Vinod, Kher, Ravi and Mahital Jamwa (2009). Effect of fym, urea and *Azotobacter* on growth, yield and quality of strawberry Cv. *Chandler L. Not. Bot. Hort. Agrobot. Cluj* **37** (1), 139-143.

Vipin Kumar and Gurdeep Singh (2010). Efficacy of fly –ash based biofertilizer vs perfected chemical fertilizer in wheat (*Triticum aestivum*). *Middle-East J. Sci. Research* **6** (2): 185-188.

V. Zecevic, J. Boskovici, M. Dimitrijevic and S. Petrovic (2010). Genetic and phenotypic variability of yield components in wheat (*Triticum aestivum L.*). *Bulgarian J. Agric. Sci.,* **16** (4), 422-428.

**** * ****