J. Environ. Sci. Pollut. Res. - Volume 3 Issue 1 (2017) 149–152

ISSN: 2455-0272

Share Your Innovations through JACS Directory

Journal of Environmental Science and Pollution Research

Visit Journal at http://www.jacsdirectory.com/jespr

Evaluation of Growth, Physiology and Yield of Mung Bean (*Vigna radiata)* by Inoculating Isolated Nitrogen Fixing Bacteria from Pharmaceutical Wastewater

D. Dutta*, D. Kundu, J.K. Datta

Department of Environmental Science, The University of Burdwan, Burdwan – 713 104, West Bengal, India.

ARTICLE DETAILS

Article history:
Received 17 January 2017
Accepted 26 January 2016
Available online 14 February 2017

Keywords:
Mung Bean
Pharmaceutical Effluent
Bacterial Inoculants
Soil Quality

ABSTRACT

Pharmaceutical effluents are hazardous to the environment producing enormous toxicity. But this effect can be utilized for the betterment of growth in the agricultural fields. Non-symbiotic nitrogen fixing bacteria were isolated from this effluent and used for the growth of wheat. A field work was conducted for this. This species of bacteria was used for the growth of mung bean with its two treatments along with control. A drastic change was observed after 60 DAS (Days After Sowing). There was an increase in the agricultural production, improvement in the soil properties, and increase in soil fertility. The other growth criterions like root and shoot length, root and shoot fresh and dry weight, chlorophyll content and other yield parameters were also measured, which were found to be increased by this bacterial inoculation effect. This study shows how we can use a toxic effluent for the better yield of agricultural crops and obviously it might prove to be a boon to our environment and farmers.

1. Introduction

Mung bean (*Vigna radiata* L.) is a short-duration legume crop, well known and widely-grown in south and Southeast Asia. This crop is a very important source of protein and iron and act as a substitute of animal protein in most Asian diets. It is worth mentioning that reduction in the use of inorganic fertilizer in agriculture system is very much important while pollution is a great concern. Organic agricultural management practices facilitate higher crop yield and improvement of soil quality [1].

The pharmaceutical industry manufactures wide range of biological products, medicinal chemicals, botanical products, and the pharmaceutical products which can be used as human and animal medications. Though the pollutants concentration in pharmaceutical effluents is high but it also contains many useful bacterial strains which could be utilized for different benevolent activities of the society. Biological nitrogen fixation is of great practical importance since the potential environmental hazards of nitrogenous fertilizer have raised ecological concern and also the fertilizer are becoming steadily less economic [2].

Uses of living microorganisms which are able to fix atmospheric nitrogen are very much helpful. They increase the availability of nitrogen to plants by living freely in the soil or by symbiotic association. Through the biological process they mobilize the nutritional elements from non-usable to usable [3]. The use of living nitrogen fixers assist to diminish chemical nitrogen fertilizer and therefore improve plant growth as well as yield attribute and decrease environmental risk. Use of nitrogen fixing bacteria as fertilizer in non-legume crops production has a great importance in recent years [4].

The present study is based on the hypothesis, that application of nitrogen fixing bacteria may improve the soil quality and yield of the mung bean. Hence, this investigation was undertaken to determine the effect of application of nitrogen fixing bacteria isolated from pharmaceutical wastewater on the growth, physiology and yield of mung bean as well as soil fertility and health under the agro-climatic conditions of the old alluvial soil zone of Burdwan, West Bengal, India.

*Corresponding Author
Email Address: deblina69envs@gmail.com (D. Dutta)

2. Experimental Methods

2.1 Geographical Location of the Experimental Area

Experiment was performed at the Crop Research and Seed Multiplication Farm, The University of Burdwan, Burdwan, West Bengal, India. The latitude and longitude of the experimental area is 23°15'5.44" N and 87°50'35.79" E and elevation 53 m respectively (Fig. 1). This area has a monsoon of subtropical climate with three distinct season, viz, Rainy (July to October), winter (November to February), and summer (March to June). The mean annual precipitation is about 1141.7 mm. Mainly, the precipitation is high in rainy season about 84%. The maximum and minimum temperature fluctuated between – 23.8 °C to 36.8 °C and 9.1 °C to 26.4 °C respectively. The experiment was carried out during the winter season in the year of 2011-2012.

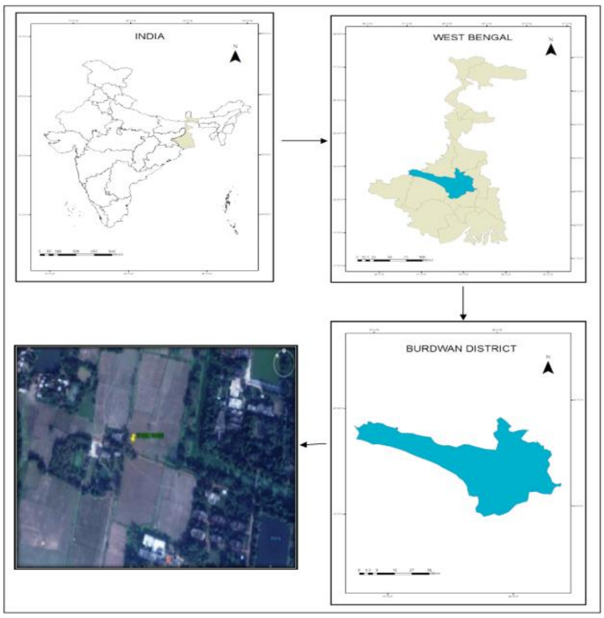

Fig. 1 Experimental area

2.2 Isolation of Bacteria from Pharmaceutical Effluent

About 1 mL of sample water was dissolved in 9 mL of sterile glass distilled water and kept in a test tube in a test tube stand. 1 mL of sample water solution was added to 9 mL of sterile distilled water to give a dilution of 10^{-1}. Similarly, 1 mL of solution from this test tube was transferred to 9 mL sterile distilled water in the next tube to prepare a dilution of 10^{-2}. Thus, dilution of 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were prepared. About 25 mL of molten Agar (Ashby's Glucose Agar) was plated in petri dishes and 1 mL of water sample from each dilution was added. The plates were gently shaken so as to spread the water sample uniformly on the medium. The inoculated plates were incubated at 37±1 °C for 24-48 hrs. Then the bacterial colonies were formed.

2.3 Treatments

The treatments used were as follows:
Control T1 – Recommended dose of chemical fertilizer
(58.32 kg/hectare nitrogen + 75 kg/hectare phosphate)
T2- Recommended dose of chemical fertilizer + 10^5 CFU/mL inoculums
T3- Recommended dose of chemical fertilizer + 10^8 CFU/mL inoculums

Experiment was carried out in a Randomized Block Design (RBD) and all treatments were replicated thrice. Sizes of each plot were 4 × 2.5 m^2 where respective treatments were applied. Row-to-row and plant-to-plant spacing was 30 cm and 15 cm respectively. Proper irrigation channel were implemented to ensure easy and uninterrupted flow of irrigation for each individual plot.

2.4 Crop Sowing

Previously soaked seed (24 h) in distilled water were mixed with nitrogen fixing bacteria and were sown separately in (4×2.5 m^2) plots during December, 2011 and carried out upto February 2012. Inoculation was carried out by dipping the seeds in the cells suspension of 10^5 and 10^8 CFU/mL for 15 min. Weeds were removed manually at 15-18 DAS (Days After Sowing) and 38-40 DAS. The crops of each plot were harvested separately when 90% of the plants become pale yellow in color.

2.5 Collection of Data

Different parameters like growth attribute, morphology, yield and biochemical analysis of plants were recorded at different stages, i.e., 30 DAS, 45 DAS, 60 DAS of crop growth as documented as below.

2.5.1 Growth Parameters

Root length, shoot length, fresh weight root, fresh weight shoot, fresh weight leaf, dry weight root, dry weight shoot and dry weight leaf were taken under consideration for the measurement of the growth parameter. 5 plants were randomly taken from each plot for measurement. Shoots length were measured from ground base to top of the plant and roots length after careful uprooting the plants, using a scale.

2.5.2 Morpho-Physiological Parameters

Observations on morpho-physiological traits, such as leaf area index (LAI) [5], leaf area ratio (LAR), leaf area duration (LAD), crop growth rate (CGR) [6], net assimilation rate (NAR) [7], and Relative Growth Rate (RGR) were recorded at different stages of crop development. Calculations for different parameters measurement involve in the morpho-physiological character are addressed below:

LAI = Leaf area/land area

$$\text{LAR} = \frac{\text{LA}}{\text{W}}\ \text{m}^2/\text{g}$$

Where, LA = leaf area in m^2 and W = dry weight of plant.

$$\text{LAD} = \frac{(\text{LAI}_1 + \text{LAI}_2)\times(t_2 - t_1)}{2}\ \text{days}$$

Where, LAI_1 and LAI_2 are leaf area indices recorded at times t_1 and t_2, respectively.

$$\text{NAR} = \frac{W_2\text{-}W_1/t_2\text{-}t_1}{L_2\text{-}L_1/\log_e L_2\text{-}\log_e L_1}\ \text{g/m}^2\text{/day}$$

Where, W_2 and W_1 are the final and initial dry weights of aerial plant parts per unit area at the times t_1 and t_2 respectively, and L_2 and L_1 are the final and initial leaf indices at respective times.

$$\text{CGR} = \frac{W_2\text{-}W_1}{t_2\text{-}t_1}\ \text{g/m}^2\text{/day}$$

Where, W_2 and W_1 are the final and initial total dry weights of above ground plant parts per unit land area (m^2) at the consecutive time t_1 and t_2 respectively.

2.5.3 Yield Component, Their Associate Characters and Yield

For the yield measurement different parameters like number of pods per plant, number of grains per pod, weight of grain, grain yield and number of branches per plant. 10 randomly selected plants of each plot were taken for the measurement of number of pod and grain per pod. For the weight measurement of grain 1000 seeds were counted separately from each treatment and discarding ring line, grain weights were recorded from the harvested plants of the net area, ear-marked for yield estimation.

2.5.4 Biochemical Parameter

Different biochemical parameters like chlorophyll [8], sugar [9] and proline [10] were estimated for plant in different stages of growth.

2.5.5 Analysis of Chemical Composition of Soil

Soil samples were collected before the experiment started and after the harvesting of the crop from 0-30 cm depth of three selected spot. Then the samples were transferred into polythene bag and taken to the laboratory for the analysis. Samples were air dried, grounded and sieved to achieve desired particle size. Different soil parameters like pH [11], organic carbon [12], available nitrogen [13], $NaHCO_3$ extractable phosphate phosphorus [14] and available potassium [15] were measured.

3. Results and Discussion

3.1 Growth Attributes

On 30 DAS highest root length was recorded with T3 and minimum with control. T2 showed result in between this two and the trend was T3>T2>T1. On 45 DAS similar trend was noted i.e., in the order of T3>T2>T1. The root growth on 60 DAS i.e., in the final stage of growth also showed the similar trend as observed with 30 DAS and 60 DAS. From the result, it appears at any stage of root growth i.e., on 30 DAS, 45 DAS and 60 DAS, the bacteria obtained from pharmaceutical waste had some positive influence on the root growth over control. Since, root growth is an important factor for crop physiological as well as yield and also its capacity of water uptake from the soil the use of indigenous resource i.e., pharmaceutical wastewater could be a potential tool for crop improvement with sustainable habit. Though, initially highest shoot length was observed in T2 on 30 DAS but in the final stage of growth T3 showed highest value. Throughout the whole experimental period highest shoot length and highest fresh weight of shoot, was observed in T3. On 30 DAS highest fresh weight of leaf was recorded with T2 and minimum with control (T2>T3>T1). On 45 DAS the trend was noted in the order of T1>T2>T3. The fresh weight on in the final stage of growth showed the trend as T3>T1>T2. In the case of dry weight of root T2 showed highest value at the final stage of growth. Relative water content showed the trend as T3>T2>T1 at the end of the experiment. All the results have been presented in Table 1.

Table 1 Changes in growth attributes of mung beans under recommended dose of chemical fertilizer and bacterial inoculation with different time interval

Parameters	30 DAS			45 DAS			60 DAS		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Root length (cm)	9.00	9.46	10.2	12.4	12.7	14.2	15.9	17.9	19.9
Shoot length (cm)	28	30.5	28.5	45.1	39.9	39.8	39.2	43.7	45.5
Fresh weight (Root) (g)	0.33	0.5	0.51	1.59	1.65	1.36	1.60	2.09	2.22
Fresh weight (Shoot) (g)	2.48	3.04	4.45	11.8	14.0	12.6	12.7	11.0	14.4
Fresh weight (Leaf) (g)	2.06	2.67	2.51	11.7	10.2	7.57	8.54	8.20	12.9
Dry weight (Root) (g)	0.13	0.19	0.24	0.46	0.48	0.45	0.81	1.01	0.73
Dry weight (Shoot) (g)	0.49	0.71	0.96	2.51	2.83	2.65	3.74	4.63	4.91
Dry weight (Leaf) (g)	0.52	0.78	0.74	2.52	3.19	1.69	2.23	2.21	2.22
Relative water Content (%)	54.4 99	39.9 59	38.3 49	76.8 42	55.6 63	69.3 58	54.1 52	76.5 71	90.6 58

3.2 Morpho-Physical Parameters

Though, at 30 DAS, highest LAI was recorded with T2 but at the middle and maturity stage of the cultivation the trend was T3>T2>T1. On 45 DAS and 60 DAS highest LAR was recorded in T3 and the trend was T3>T1>T2 in the final stage of growth. The time span of active photosynthesis by leaves is represented by LAD. Both the treatment showed significant effect over control. During 45-60 DAS the highest LAD was recorded with T3 and minimum with T1.T2 showed result in between this two and the trend was T3>T2>T1. During the time span of 45-60 DAS the highest CGR and NAR were recorded with T3 and the trend was T3>T2>T1. All the results have been presented in Table 2.

Table 2 Changes in morpho-physical parameters of mung beans under recommended dose of chemical fertilizer and bacterial inoculation with different time interval

Parameters	30 DAS			45 DAS			60 DAS		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
LAI (m^2g^{-1})	0.499	0.778	0.713	0.574	0.873	1.091	0.570	0.871	1.084
LAR	0.012	0.009	0.008	0.003	0.002	0.003	0.003	0.003	0.004
CGR (g^2 day^{-1})	3.23	4.79	5.85	4.141	5.25	9.093	10.13	10.87	11.28
LAD (days)	6.97	8.45	8.89	8.033	12.37	13.475	8.593	13.103	16.313
NAR (g^2 day^{-1})	0.653	1.989	4.356	0.7403	2.165	5.482	4.312	5.071	8.34

3.3 Yield Attribute

In initial phase i.e., 30 DAS and 45 DAS as plants were not so developed hence, there were no yield attribute in all treatments. Different yield parameters like pods per plant, number of pods per plant, pod + grain weight and weight of 100 seeds of the plant were maximum in T3. All the results have been presented in Table 3.

Table 3 Changes in yield attributes of mung beans under recommended dose of chemical fertilizer and bacterial inoculation with different time interval

Parameters	30 DAS			45 DAS			60 DAS		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Pods/Plant	0	0	0	0	0	0	20.67	21	23.33
Grains/Pod	0	0	0	0	0	0	5.9	6.2	6.3
Pod+Grain wt. (g)	0	0	0	0	0	0	976.6	1037	1321
Only Pod (g)	0	0	0	0	0	0	89.83	113.3	145
1000 Seed wt. (g)	0	0	0	0	0	0	24.95	28.87	30.71

3.4 Biochemical Parameters

The chlorophyll was measured as chlorophyll a, b and total chlorophyll. On 30 DAS the trend of chlorophyll a, b and total chlorophyll was also T3>T2>T1.On 45 DAS the chlorophyll has been found to be greater than 30 and 60 DAS. The trend of chlorophyll a, b and total chlorophyll was also the same i.e., T3>T2>T1.On 60 DAS the result was found to be less than the result of 45 DAS but the trend was remain same. On 45 DAS and 60 DAS, the chlorophyll content in the pods were also measured. The trend of the results of both DAS were found to be as T3>T2>T1. The results of 45 DAS are greater than that of 60 DAS. On 30 DAS highest sugar, protein, proline and ascorbic acid were recorded with T3 and minimum with control. On both 45 DAS and 60 DAS the trend was T3>T2>T1 and the results of 45 DAS were greater than 30 and 60 DAS. All the results have been presented in Table 4.

Table 4 Changes in biochemical parameters of mung beans under recommended dose of chemical fertilizer and bacterial inoculation with different time interval

Parameters	30 DAS			45 DAS			60 DAS		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Chlorophyll a leaf ($mg\ g^{-1}$ FW)	0.10	0.10	0.11	0.26	0.29	0.30	0.18	0.21	0.22
Chlorophyll b leaf ($mg\ g^{-1}$ FW)	0.05	0.06	0.10	0.26	0.31	0.33	0.13	0.16	0.20
Total Chlorophyll leaf ($mg\ g^{-1}$ FW)	0.12	0.16	0.17	0.57	0.57	0.62	0.40	0.42	0.48
Chlorophyll a pod ($mg\ g^{-1}$ FW)	0	0	0	0.07	0.31	0.32	0.04	0.06	0.07
Chlorophyll b pod ($mg\ g^{-1}$ FW)	0	0	0	0.06	0.37	0.49	0.02	0.03	0.03
Total Chlorophyll pod ($mg\ g^{-1}$ FW)	0	0	0	0.12	0.61	0.73	0.07	0.07	0.10
Sugar (mg/g)	1.14	1.51	1.87	2.37	3.38	3.97	7.67	9.83	12.7
Proline (mg/g)	5.72	6.57	9.39	14.9	16.2	17.7	11.8	20.1	26.9

FW- Fresh weight

3.5 Chemical Composition of Soil

Soil alkalinity was increased by 1.10 fold after harvesting of the crop where conductivity was decreased at the end and T1 showed the highest conductivity followed by T2 and T3. Organic carbon content was increased by 2.16 fold after harvesting the crop where T3 showed the highest value (8.04 %). Three nutrient parameters like N, P and K were also increased by 1.19, 1.02 and 1.004 fold respectively. All the results have been presented in Table 5.

Table 5 Changes of soil properties in a mung cropping system before sowing and after harvesting

Parameters	Before sowing	After harvesting		
		T1	T2	T3
pH	6.78	7.19	7.48	7.35
Conductivity ($mho\ cm^{-1}$)	0.08	0.06	0.05	0.03
Organic Carbon (%)	3.72	6.86	7.88	8.04
Available Nitrogen ($kg\ ha^{-1}$)	238.336	278.45	288.68	298.99
Available Phosphorus ($kg\ ha^{-1}$)	230.56	232.44	233.58	235.24
Available Potassium ($kg\ ha^{-1}$)	228.48	228.66	229.03	229.46

In the present investigation, recommended dose of chemical fertilizer + 10^8 CFU/mL inoculums (T3) gave better results than other treatment and control. The improved growth, yield and morphology of mycorrhizal plants is attributed to a higher nitrogen fixing bacteria colonization, which is known to improve plant growth by increasing nutrient uptake and plant growth promoting substances in the rhizosphere of mung bean [16]. The present experimental result was derived from the improvement of nitrogen fixing bacteria activities in soil.

All the growth attribute parameters showed increasing trends towards maturity stage of the crops. Among all the treatments T3 showed higher value in all parameters excluding dry weight of root and leaf.

At the initial stage of development (30 and 45 DAS) of mung beans, LAI values increased but at the final stage, little bit fall of the values were observed. Maximum LAI was found in the treatment of T3. Newly emerged leaves may increase the LAI but with time LAI decrease may be due to the leaf senescence [17, 18]. LAR is the ratio of photosynthesizing to respiring material within the plant [4]. The study reveals the highest LAR during final stage of growth in T3. Higher rate of LAD in 45 DAS was due to the higher rate of dry matter accumulation during this time period [18]. CGR represent as a valuable parameter for crop production on the basis of dry matter production rate. Highest CGR was observed in T3 during 45 DAS, may be cause by higher LAI, because higher facilitate higher light interception resulted into higher CGR and higher yield [4, 19]. Increase in NAR at the final stage is attributed by higher assimilation of nutrients and formation of siliquae, cell elongation, shoot and root development, resulted into development of CGR and NAR [18].

Number of pods per plant of mung bean was significantly influenced by N levels. Increasing nitrogen level through rhizobium species living in root nodules led to an increase in pods per plant [20]. There was little bit increase in number of grains per pod in treatments to control. It may be due to the no significant effect of nitrogen level on grains per pod. But its number may perhaps be regulated during pod developing stage by means of canopy photosynthesis and the bustle of the starting materials [21]. Results reveal that addition of growth bacteria with nitrogen source had significant effect on the mung bean yield than the non-inoculated seed (control). It may be due to the positive effect on the plant growth which leads to the development of grains as well as grain yield [22]. Rather et al., [23] reported about the enhanced yield of peas using the growth bacteria. Highest seed yield was observed in the T3 which was attributed toward the production of growth promoting substances [24].

Photosynthesis process helps to increase the productivity of crop and chlorophyll content in different segments of plant is the good indication of it [17, 18]. Chlorophyll content was showed highest value during 45 DAS and gradually decreased at the final stage of experiment. While T3 showed the highest chlorophyll content over T2 and T1. Variation in chlorophyll content in leaf and pod of different treatments may be due to the photosynthesis and biosynthesis of chlorophyll. Chandrasekhar et al., [25] also reported increased chlorophyll content by using nitrogen fixing *Azotobacter* species. Nitrogen has a positive effect on the formation of chloroplast which is clearly evidence in this experiment [26]. Difference in sugar content in different treatment may be due to the variable production of photosynthate. Uses of nitrogen fixing bacteria facilitate the biosynthesis and accumulation of sugar in plant. Similar types of finding were established by Mandal et al [27]. In this experiment gradual increment of proline content with time were observed and highest value was recorded in T3. Variable proline content in leaves reveals different response toward environmental stress and different levels of osmotic adjustment [28].

While soil quality is also most vital, it is worth mentioning that use of bacterial inoculation enhanced the biochemical property of the soil. Soil pH become slight alkaline after harvesting of crops compared to soils before sowing [27] and T2 showed the highest value. Use of bacterial inoculation along with chemical fertilizers treatments had conductivity, among which T3 showed the highest conductivity value. This progressive increase in the level of conductivity in all treatments may be attributed to the combined effect of inoculation and chemical fertilizer which have contributed to the increase in buffering capacity of the soil. Acids from organic matter decomposition reacted with sparingly soluble salts which are present in the soil, either converted them into soluble salts or increased their solubility hence, increasing the conductivity of the soil [29]. The percentage of organic carbon of the field after harvesting was gradually build-up in which microbial inoculation and chemical fertilizers were added.

Decomposition and conversion of complex organic matter in composts and mineralized organic colloids respectively, took place, and thus added to the soil carbon pool. The results of the present investigation are in agreement with the findings of Mondal et al [27]. The results showed that the concentration of the available N in soil was affected by inoculums and chemical fertilizer treatment. T3 showed the maximum available N compared to T1, which produced more residual N in soil than in control. Higher concentration of available N in soil after harvesting than before sowing were due to the positive interaction among applied microbial community and soil components through biological fixation. The results of the present investigation are in agreement with the previous findings [29]. There were not too much increased in phosphorus and potassium concentration in the soil after harvesting of the crops. It may be due to higher amounts of crop uptake due to higher mobilization of phosphorous and potassium in soil from bound form to available form.

4. Conclusion

The present study reveals the enhanced growth pattern, morpho-physiological characteristics and yield attributes, with the application of pharmaceutical waste generated bacterial inoculation under such agro-climatic zone. Variable biochemical responses resulted into the accumulation of chlorophyll, sugar and proline content in the crop. Since, this is an eco-friendly technology originated indigenously from a waste material this is indeed a very useful tool for utilization of waste as well as enhanced crop production through its benevolent activities for the living beings. Present treatment combination could be recommended to the end users and further studies on this aspect on other crops will make farmer's community for getting a low cost product with healthy atmosphere for crop generation.

References

[1] Pedramminaee, Md.R.H.S. Hadi, Md.T.D.A.M. Shahsavar, Effects of nitrogen fixing bacteria and amino acids spraying on yield and yield components of mung bean (*Vigna Radiate*), Ann. Biol. Res. 4 (2013) 265-269.

[2] J.I. Sprent, P. Sprent, Nitrogen fixing organisms: pure and applied aspects, Chapman and Hall, London, 1990.

[3] K. Dhevendaran, G. Preetha, N. Bodethala, V. Hari, Studies on nitrogen fixing bacteria and their application on the growth of seedling of *Ocimum sanctum,* Pharmacogn. J. 5 (2013) 60-65.

[4] T. Mondal, J.K. Datta, N.K. Mondal, Chemical fertilizer in conjunction with biofertilizer and vermicompost induced changes in morpho-physiological and bio-chemical traits of mustard crop, J. Saudi Soc. Agric. Sci. (2015) DOI: 10.1016/j.jssas.2015.05.001.

[5] D.J. Watson, Comparative physiological studies on growth of field crops, I variation in net assimilation rate of leaf area between species and varieties and within years, Ann. Bot. 11 (1947) 141-146.

[6] R. Hunt, Plant growth analysis studies in biology, Edward Arnold, London, 1978.

[7] D.J. Watson, The physiological basis of variation in yield, Adv. Agron. 4 (1952) 101-145.

[8] D.I. Arnon, Copper enzyme polyphenoloxides in isolated chloroplast in *Beta vulgaris*, Plant. Physiol. 24 (1949) 1-15.

[9] R.M. McCready, J. Guggolz, V. Silviera, H.S. Ownes, Determination of starch and amylase in vegetables, application to peas, Anal. Chem. 22 (1950) 1156-1158.

[10] L.S. Bate, R.P. Waldren, L.D. Teare, Rapid determination of free proline for water stress studies, Plant. Soil. 39 (1973) 205-207.

[11] M.L. Jackson, Soil chemical analysis, Prentice Hall of India Pvt. Ltd, New Delhi, 1972.

[12] D. Walkley, C.A. Black, An examination of Degtareff methods for determining soil organic matter and proposed modification of the chronic and a proposed modification of the chromic acid titration method, Soil Sci. 37 (1934) 29-38.

[13] B.V. Subbiah, G.L. Asija, Rapid procedure for the estimation of available nitrogen in Soil, Curr. Sci. 25 (1956) 259-260.

[14] C.A. Black, Methods of soil analysis, Part 1. Physical and mineralogical, 1965.

[15] S.R. Olsen, C.V. Cole, F.S. Watnabe, L.A. Dean. Estimation of available phosphorous in soils by extraction with sodium bicarbonate, U.S. Department of Agriculture Circular, USA, 1954.

[16] N. Kadian, K. Yadav, A. Aggarwal, Bioassociative effect of rhizospheric microorganisms on growth, nutrient uptake and yield of mung bean (*Vigna radiata* L. Wilczek), Eur. J. Environ. Sci. 3 (2013) 27-34.

[17] A. Banerjee, J.K. Datta, N.K. Mondal, Biochemical changes in leaves of mustard under the influence of different fertilizers and cycocel, J. Agric. Technol. 8 (2012) 1397-1411.

[18] A. Banerjee, J.K. Datta, N.K. Mondal, Changes in morphophysiological traits of mustard under the influence of different fertilizers and plant growth regulator cycocel, J. Saudi Soc. Agric. Sci. 11 (2012b) 89-97.

[19] J.K. Datta, A. Ghosh, A. Banerjee, N.K. Mondal, Biochemical response of selected plant species under air pollution stress, Ecol. Environ. Conserv. 18 (2012) 957-962.

[20] M.S. Anjum, Z.I. Ahmed, C.A. Rauf, Effect of Rhizobium inoculation and nitrogen fertilizer on yield and yield components of mungbean, Int. J. Agric. Biol. 8 (2006) 238-240.

[21] M.S. Akther, Physiological differences in yielding ability of traditional and modern mungbean genotypes (*Vigna radiata* (L) Wilczek), A Ph.D. thesis, Department of Agronomy, BSMRAU, Gazipur, 2005.

[22] S.M.A. Azar, A. Golchin, K.H. Besharati, Effect of irrigation, nitrogen and inoculated with Rhizobium bacteria on lentil yield in dry land conditions, Twelfth Crop Science Congress, Islamic Azad University of Karaj, Iran, 2012.

[23] S.A. Rather, M.H. Hussain, M.L. Sharma, Effect of biofertilizers on growth yield and economics of field pea (*Pisum sativum* L.), Int. J. Agr. Sci. 6 (2010) 65-66.

[24] A. Banerjee, J.K. Datta, N.K. Mondal, Impact of different combined doses of fertilizers with plant growth regulators on growth, yield attributes and yield of mustard (*Brassica campestris* cv. B9) under old alluvial soil of Burdwan, West Bengal, India Front. Agric. China 4 (2010) 341-351.

[25] B.R. Chandrasekhar, G. Ambrose, N. Jayabalan, Influence of biofertilizer and nitrogen source level on the growth and yield of *Echinochloa frumentacea* (Roxb.) Link, J. Agric. Technol. 1 (2005) 223-234.

[26] M. Singh, M.M.A. Khan, M. Naeem, Effect of nitrogen on growth, nutrient assimilation, essential oil content, yield and quality attributes in *Zingiber officinale* Rosc, J. Saudi Soc. Agric. Sci. 15(2) (2014) 171-178.

[27] T. Mondal, J.K. Datta, N.K. Mondal, Influence of indigenous inputs on the properties of old alluvial soil in a mustard cropping system, Arch. Agron. Soil. Sci. 61 (2015) 1319-1332.

[28] L. Ozturk, Y. Demir, In vivo and in vitro protective role of proline, Plant Growth Regul. 38 (2002) 259-264.

[29] A. Banerjee, J.K. Datta, N.K. Mondal, T. Chanda, Influence of integrated nutrient management on soil properties of old alluvial soil under mustard cropping system, Commun. Soil Sci. Plant Anal. 42 (2011) 2473-2492.