Int.J.Curr.Microbiol.App.Sci (2020) *9*(3): 2759-2767

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 3 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.903.316

Response of French Bean (*Phaseolus vulgaris* L. cv. Arka Arjun) to Rhizobium Inoculation under Varied Levels of Nitrogen and Molybdenum

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Keywords

French bean, Rhizobium, Nitrogen, Molybdenum, Yield attribute

Article Info

Accepted:
22 February 2020
Available Online:
10 March 2020

ABSTRACT

A study was conducted on French bean (*Phaseolus vulgaris*) at the coastal saline zone of West Bengal during winter 2017-18 and 2018-19. The soil texture was clay loam with pH 7.03. A location specific trial was done to standardize the combination dose for French bean towards productivity enhancement. The experiment was laid out in randomized bock design having 3 replications with 12 different treatments. The investigation was conducted with two strains of Rhizobium species -R (Rhizobium V_1B/Bean-15 & Rhizobium V_1B/F.bean-21) interacting on three applied levels of nitrogen-N (75%, 50%, 25% of the recommended dose @ 40kg/ha), and two levels of ammonium molybdate-Mo (50%, 100% of the recommended dose @30kg/ha) in variety "Arka Arjun" of French bean. The study generated information on the combined effects of biofertilizer and chemical fertilizer on several morphological attributes, protein content, nodulation and their final culmination on the yield. Studies on changing soil profile with respect to available nitrogen and population count of nitrogen fixers were also worked out to support the results. The mean sum of squares for all the traits were found statistically significant. The conclusive study suggests that the following treatment combinations viz. Rhizobium V_1B/bean-15 +Nitrogen 50 % +Molybdenum 100 % and Rhizobium V_1B/bean-15 +Nitrogen 25 % + Molybdenum 50 % applied to the French bean cv. Arka Arjun may be advised to the farmers of Coastal Saline Zone of West Bengal for productivity enhancement and maintaining soil sustainability.

Introduction

French bean (*Phaseolus vulgaris* L.) is a pulse crop which is rich in vitamins and minerals like calcium, iron, magnesium, phosphorus, potassium, zinc etc. It is a short duration high yielding grain legume crop that can be used both as pulse and vegetable French bean is consumed as immature tender pods, green grains are consumed as vegetables and dry grains are a good source of protein. French bean also depicts some medicinal properties that include control of diabetes, cardiac problems and natural cure for bladder burn.

As per the FAO estimates, it is grown in the world in an area of 28 million hectares with an annual production of 20 million tons and productivity of 729 kg/ha (Prakash and Ram, 2014). Foliage of the crop may also provide hay, silage and green manures and plants can be fed to cattle after harvest (Kakon *et al*., 2016).

Agronomical practices play a pivotal role to exploit the genetic worth of a crop for obtaining good production. Different growth and yield attributing traits are highly affected by the nutrient status of the growing media. French bean unlike other pulses has determinate type nodules and thus nitrogen fertilizers are to be applied in lower quantity than other pulses. Nitrogen deficiency in most crops constraints leaf area expansion, enhances leaf senescence, inhibits photosynthetic rate and consequently reduces the crop productivity (Machler *et al*., 1988 and Wolfe *et al*., 1988).

Bean legumes are capable of fixing atmospheric nitrogen by the presence of Rhizobium bacteria at their root nodules. Molybdenum is an essential element that acts as an essential cofactor in stimulating N-fixing activity of *Phaseolus vulgaris* (Vakhaniya *et al.*, 1990, Reyes *et. al*., 2016). It is also essential for certain nitrogen transformations in microorganisms as well as in plants. Molybdenum increases nitrogenous activity and extends the period of high nitrate reductase activity, with a consequent increase in total shoot nitrogen.

The main effect of Mo fertilizer application is to increase the size of nodules and avoidance of nodule senescence, therefore maintaining a longer period of effective nitrogen fixation (Vieira *et al*., 1998). Molybdenum is important and has special significance due to its contribution in activation of several enzyme systems and physiological activities within the plant body. Molybdenum is a constituent part of the enzyme nitrate reductase concerned with the reduction of nitrate to nitrite in both microorganisms and higher plants (Lal *et al*., 2018).

Declining soil fertility and high cost of fertilizer are major limitations to crop production. Requirements for nitrogen exceed any other major nutrients needed for agriculture and rarely does soil have enough of this nutrient to produce high sustainable yields (Mkandawire *et al*., 1998, Woldeyohannes *et al*., 2007).

Reduced dependence on nitrogenous fertilizer and adaptation to farming practices that favour more economically viable and environmentally prudent nitrogen fixation will benefit both agriculture and the environment (Woldeyohannes *et al*., 2007, Zaman-Allah *et al*., 2007). Biological Nitrogen Fixation (BNF) has been used in farming systems to reduce the fertilizer expenses and inoculation with an effective and persistent rhizobium strain has several advantages that include non-repeated application of nitrogen fertilizers and higher pod yield due to increase in nodulation (Otieno *et al*., 2009).

The present investigation was conducted to assess the interaction of two strains of Rhizobium species on three different applied levels of nitrogen and two levels of ammonium molybdate in variety Arka Arjun of French bean to generate information of its combined effects on several morphological attributes, protein content, nodulation and its final impact on the yield. Assessment on changing soil profile with respect to available nitrogen and population count of nitrogen fixers were also worked out.

Materials and Methods

The present field experiment was carried out at the Agricultural experimental farm of Calcutta University at Baruipur,24–Parganas(South) [88°28′ East longitude, 22°22′ North latitude] during winter season of 2017-2018 and 2018- 2019. Topography of the land was uniform with medium fertility and clay loam texture. The physicochemical characteristics of the soil were determined by following the standard methods and are being presented in Table 1.

The experiment was laid out in a randomized bock design having three replications with 12 different treatments. The size of the plot was 4 x 1 m^2 and bund width between plots was 0.26 m with 1m of irrigation channels. Net area of the experiment was 210 m^2 with a spacing of R to R 20cm, P to P 20 cm. The French bean variety seeds (*Phaseolus vulgaris* L. cv Arka Arjun) were collected from Indian Institute of Horticultural Research, Bengaluru. The bean cultivars were raised under three levels (75 %, 50 % and 25 %) of recommended nitrogen dose in the form of Urea.

Phosphorous and Potassium were applied in the form of Single Super Phosphate and Murate of Potash following the recommended dose of fertilizer N:P:K (40:60:50). Micronutrient Molybdenum was applied at two levels viz. $Mo_1$and Mo_2 @ 50% and 100% of recommended 'Mo' dose. Two microbial inoculants namely R_1-Rhizobium V_1B/Bean-15 and R_2- Rhizobium V_1B/F Bean-21 strains used for the seed treatments during the study were collected from Vivekananda Institute of Bio technology, Nimpith, West Bengal. The moist seeds were thoroughly mixed with the inoculant under the shade and were placed in the furrows at the recommended spacing of plots.The plants were thinned to one plant per hill after emergence.

The plants were thinned to one plant per hill after emergence. The treatment details of the study are: T_1 (R_1,N_1,Mo_1), T_2 (R_1,N_2,Mo_1), T_3 (R_1,N_3,Mo_1), T_4 (R_1,N_1,Mo_2), T_5 (R_1,N_2,Mo_2), T_6 (R_1,N_3,Mo_2), T_7 (R_2,N_1,Mo_1), T_8 (R_2,N_2,Mo_1),T_9 (R_2,N_3,Mo_1), T_{10} (R_2,N_1,Mo_2), T_{11} (R_2,N_2,Mo_2), T_{12} (R_2,N_3,Mo_2) and T_{13} (Control). (R_1-RhizobiumV$_1$B/bean-15,R_2-RhizobiumV$_1$B/F.bean-21, N_1-Nitrogen 75 %, N_2- Nitrogen 50 %, N_3-Nitrogen 25 %, Mo_1-Molybdenum 50 %, Mo_2- Molybdenum 100 %.

Five random plants were selected from the plots and 50% flowering, height of the plants, leaf count and number of branches were recorded from the selected plants on the 70th day after sowing. Leaf area was determined from fresh and matured leaves collected from the mid portion of the plant and the data was recorded by utilizing a Leaf area meter (Makeup: Systronics, Model No: 211). Fresh and dry weight of shoots and roots were taken independently on a single pan electrical balance. Yield attributes like average pod weight, number of fruits per plant and number of seeds per pod were recorded from the selected plants.

Dry weight of pod was calculated on the basis of 100 g of fresh weight per treatment plot. Yield per hectare was calculated by converting average yield obtained from each plot representing the treatments under three replicates and up scaled to the predictable yield. Number of nodules of five random tagged plants were counted and fresh weight of the nodules was recorded by using a precision electronic balance (Makeup: Kern, Model: 440-21N).Oven dry weight of the fresh nodules after attaining a constant weight at 50-52^{0}C were also recorded using a precision electric balance. Estimation of the amount of protein in the pods was carried out by following Lowry's method (Lowry *et al*., 1951).

Total number of nitrogen fixers was counted by recording the colony forming unit (CFU) on Jensen's agar plates grown from serially diluted soil samples after incubation for 72hrs at 28°C (Aneja,2003). Available nitrogen in the soil at pre and post experimentation was estimated by following the method of Jackson (2005).

Plot means of one pooled data over two seasons were used for statistical analysis. A three way factorial design was followed for interpretation of the results, Factor A representing two Rhizobium levels, Factor B representing three N levels applied in the form of Urea while Factor C represented the two molybdenum levels in the form of Ammonium molybdate.

The entire analysis was carried out at the Department of Horticulture, Institute of Agricultural Science, University of Calcutta using statistical package "Three factor Factorial CRD" software available online.

Results and Discussion

Growth characters

Beneficial effect of the combined application of rhizobium culture with nitrogen fertilizer and molybdenum on growth and development of French bean has been clearly brought out in this investigation.

Application of 25% nitrogen and 50% molybdenum along with rhizobium $V_1b_/$Bean15 showed a notable increase in growth parameters like plant height, leaf area, number of leaves, shoot and root fresh and dry weight than control (Table 2). It seemed that molybdenum played a major role either in combination with nitrogen or the strains of rhizobium to increase plant height (Masanta and Biswas,2009).

Leaf area and number of leaves as influenced by rhizobium inoculants and different levels of nitrogen and molybdenum showed significant increase for the trait. The synergistic effect between Mo and P fertilizers may have influenced better growth and development of plants (Kandil *et al*., 2013).

There was a significant increase in number of branches along with shoot fresh and dry weight. Increase in root fresh and dry weight was also recorded that was a consequent result of increase in plant height due to application of Mo and P fertilizers (Maingi *et al*., 1999 and Kandil *et al*., 2013).

Yield and yield attributes

Activity of rhizobium resulted in significant increase in number of pods per plant with highest pod length and diameter on application of 100 % molybdenum in combination with R_1 V_1B/bean15 (Table 3).

This was mainly due to the availability of the microorganism to fix atmospheric nitrogen to soil which was made available to the growing plants beside secretion of growth promoting substances which were partly responsible for the enhanced plant growth and yield. Reyes *et al.*, (2016) revealed that inorganic N fertilization and rhizobium inoculation with additional molybdenum probably increased nitrate reductase activity which in turn increased pod yield.

Pod fresh and dry weight also increased during the study which suggests that application of inorganic N may be sometimes better over biological N_2 fixation alone (Beshir *et al*., 2015). More over French bean has greater mineral N uptake efficiency as compared to N_2 fixation (George and Singleton, 1992).

This may indicate that French beans early end demand could have been satisfied from the applied N source than N from fixation that have contributed to the increase in pod fresh weight at a later stage.

Table.1 Soil parameter analysis

Mechanical Analysis	Physical Analysis	Chemical Analysis
Sand - 25% **Silt - 35%** **Clay - 40%**	a) Apparent density -1.24gm/cc b) Absolute specific gravity -2.56 c) Maximum water holding capacity - 53.80%	a) Soil pH –7.03 b) EC- 329 milliohms' c) TDS-241ppm. d) Salt-156ppm. e) Available nitrogen- 54.786 kg/ha f)Available phosphorus-30kg/ha g) Available potassium-260kg/ha.

Table.2 Effect of Rhizobium, nitrogen and molybdenum treatments on morphological traits of *Phaseolus vulgaris* L. (Pooled data of 2 years)

Treatment	Plant height (cm)	Leaf Area (cm^2)	Number of Leaves/Plant	Number of Branches/Plant	Shoot Fresh Weight(g)	Shoot Dry Weight(g)	Root Fresh Weight(g)	Root Dry Weight (g)
T_1	36.91	64.50	42.00	5.83	34.13	14.48	14.48	5.75
T_2	38.63	60.11	36.66	6.16	39.53	14.14	14.14	6.79
T_3	41.22	81.96	42.00	5.96	41.83	13.43	13.43	7.08
T_4	37.83	64.56	35.66	6.25	40.46	15.97	15.97	6.69
T_5	40.48	74.95	35.66	6.86	35.58	15.50	15.50	6.74
T_6	40.73	74.96	36.00	7.36	35.39	13.06	13.06	6.86
T_7	36.56	82.93	35.33	6.02	40.37	14.28	14.28	5.75
T_8	39.85	64.83	32.33	5.76	34.97	11.78	11.78	6.49
T_9	38.80	73.23	35.00	5.88	32.95	13.93	13.93	6.17
T_{10}	38.58	63.82	31.33	5.58	55.22	13.68	13.68	6.08
T_{11}	39.40	64.18	32.33	6.39	35.19	13.28	13.28	6.29
T_{12}	41.00	61.21	37.00	7.04	60.15	19.20	19.20	8.70
Control	24.44	60.50	27.33	4.73	25.93	10.66	10.66	4.34
CV (%)	4.82	7.94	6.27	9.67	4.92	3.27	3.27	6.42
CD .5(%)	3.19	9.32	3.81	1.02	3.37	0.79	0.79	0.72

T_1 (R1,N1,Mo1), T_2 (R1,N2,M_O1), T_3 (R1,N3,M_O1) , T_4 (R1,N1,M_O2), T_5 (R1,N2,M_O2), T_6 (R1,N3,M_O2), T_7 (R2,N1,M_O1),T_8 (R2,N2,M_O1), T_9 (R2,N3,M_O1), T_{10} (R2,N1,$M_0$2), T_{11}(R2,N2,M_O2), T_{12} (R2,N3,M_O2)
(R1- Rhizobium V_{1B}/bean-15, R2- Rhizobium V_{1B}/F.bean-21, N1-Nitrogen 75 %(315g/plot), N2- Nitrogen 50 %(210g/plot), N3-Nitrogen 25 %(105g/plot),M1-Molybdenum50 %(60mg/plot), M2- Molybdenum 100 %(120mg/plot).

Table.3 Effect of Rhizobium, nitrogen and molybdenum treatments on yield and yield related traits of *Phaseolus vulgaris* L. (Pooled data of 2 years)

Treatment	Number of Pods/Plant	Pod length(cm)	Pod diameter(mm)	Pod fresh Weight(g)	Pod dry weight (g)	Number of seeds/pod	Number of nodule/Plant	Nodule fresh weight(mg)	Nodule dry weight(mg)	Yield/ha
T_1	24.65	14.28	8.27	44.11	7.37	6.00	29.50	1073.00	332.00	4488.74
T_2	21.96	13.67	7.97	34.15	4.81	4.96	20.50	890.66	201.00	3051.96
T_3	22.66	14.48	8.33	39.67	5.99	5.46	23.50	936.33	215.00	3724.45
T_4	21.00	14.17	7.99	37.55	5.03	5.26	20.50	733.00	153.66	3121.40
T_5	25.41	14.85	8.77	44.11	6.46	5.96	35.50	1350.00	496.33	4578.69
T_6	25.21	14.66	8.56	39.99	5.90	5.83	29.50	1104.00	410.00	4170.51
T_7	21.75	14.25	8.13	32.39	5.07	5.50	20.50	897.00	186.33	2948.65
T_8	22.13	13.86	7.91	28.92	6.03	5.50	19.50	892.33	191.00	2675.14
T_9	22.38	14.48	7.97	35.16	4.87	5.13	20.50	693.00	150.33	3208.06
T_{10}	22.51	14.26	8.21	35.87	5.13	5.71	24.00	965.33	213.33	3392.31
T_{11}	23.86	14.52	8.38	41.17	6.51	6.51	32.00	1070.33	338.66	4188.93
T_{12}	23.77	14.38	8.68	38.91	7.21	6.70	26.50	1057.33	332.00	3725.38
Control	17.80	13.48	7.58	34.01	5.03	4.70	18.50	613.33	129.33	2621.2
CV (%)	3.12	2.94	4.00	3.28	7.39	2.18	7.60	6.95	6.993	4.18
CD 0.5(%)	1.22	0.71	0.56	2.09	0.73	0.21	4.21	114.41	31.67	255.72

T_1 (R1,N1,Mo1), T_2 (R1,N2,M_O1), T_3 (R1,N3,M_O1) , T_4 (R1,N1,M_O2), T_5 (R1,N2,M_O2), T_6 (R1,N3,M_O2), T_7 (R2,N1,M_O1),T_8 (R2,N2,M_O1), T_9 (R2,N3,M_O1), T_{10} (R2,N1,M_O2), T_{11}(R2,N2,M_O2), T_{12} (R2,N3,M_O2)
(R1- Rhizobium V_{1B}/bean-15, R2- Rhizobium V_{1B}/F.bean-21, N1-Nitrogen 75 %(315g/plot), N2- Nitrogen 50 %(210g/plot), N3-Nitrogen 25 %(105g/plot),M1-Molybdenum50 %(60mg/plot), M2- Molybdenum 100 %(120mg/plot).

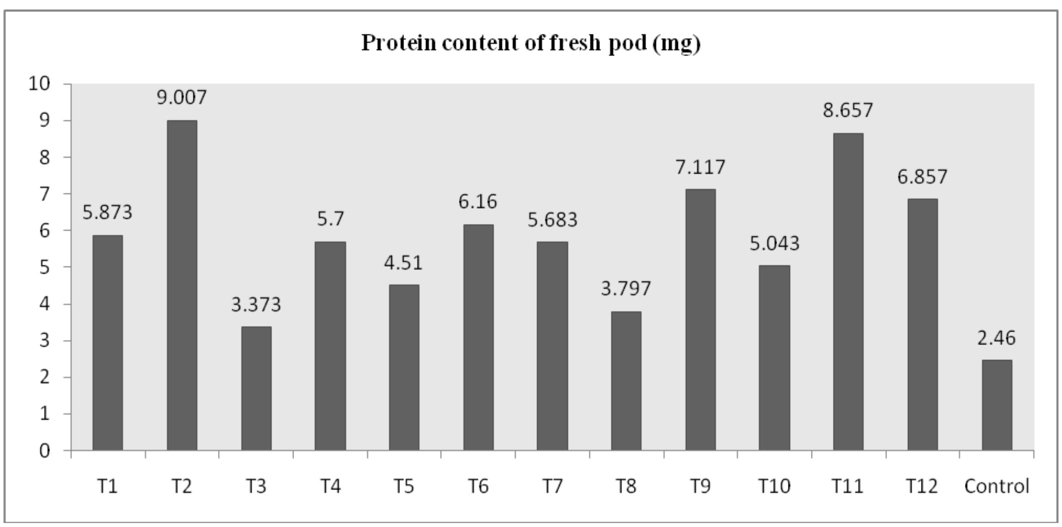

Fig.1 Protein content of fresh pods of *Phaseolus vulgaris* L. (Pooled data of 2 years)

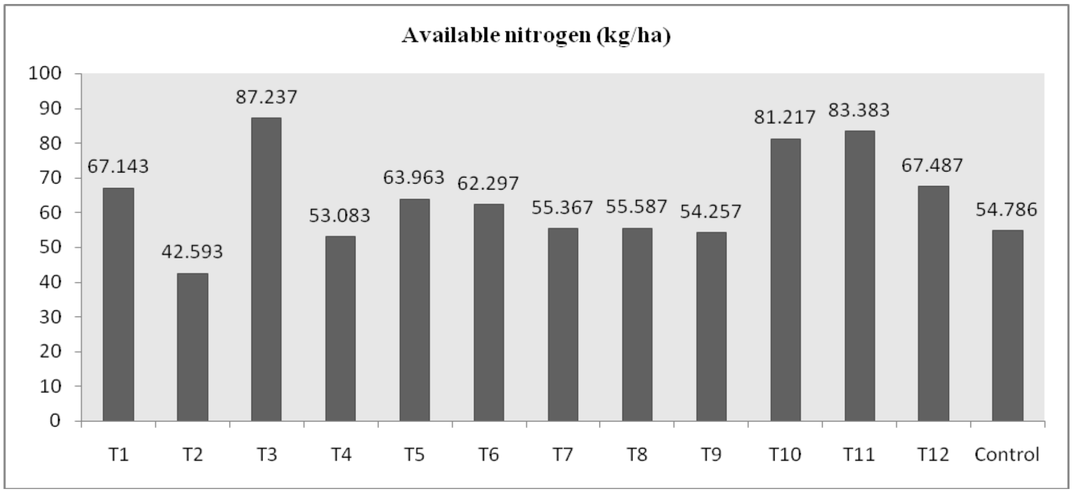

Fig.2 Available nitrogen content of soil treated with different combinations of Rhizobium, nitrogen and molybdenum (Pooled data of 2 years)

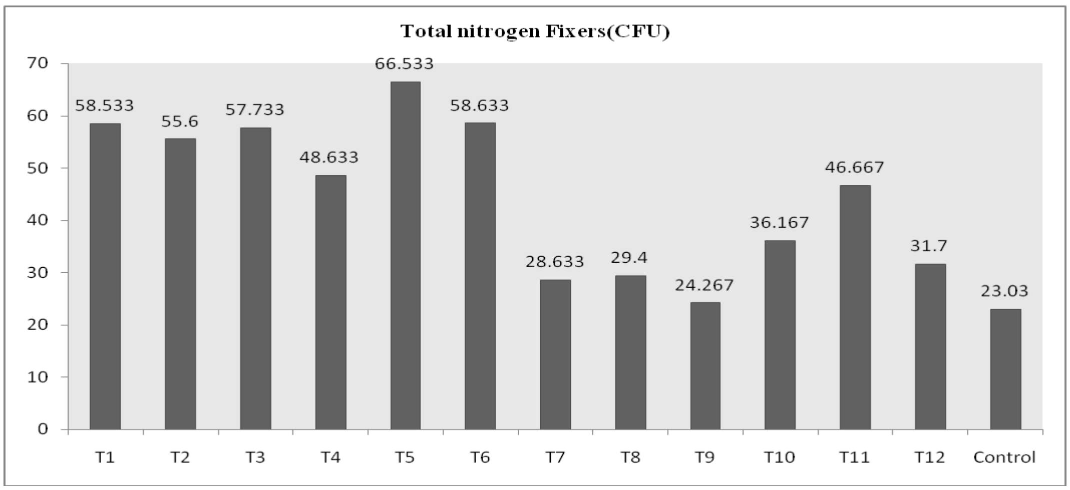

Fig.3 Total nitrogen fixers present in the soil under different treatment combinations (Pooled data of 2 years)

Increase in number of seeds per pod suggested that the inoculated bean treatments with rhizobium V_1B/F.bean-21 strain and Mo addition showed the best effect in increasing number of seeds highlighting the important role of molybdenum on nitrogenase and xanthine oxydase-de hydrogenase activity in the atmospheric N reduction and purine metabolism respectively (Marschner, 1995). Number of nodules and maximum nodule fresh and dry weight showed a significant increase during the study. Inorganic fertilization with additional Mo treatment and rhizobial inoculation showed the best effect in increasing nodulation in beans. Optimal supply of Mo to the beans inoculated with *Rhizobium etli*, increased nodule number by 88% which could be explained by the importance of Mo in symbiotic N fixation of legume, which is an important co-factor of nitrogenase complex and xanthine oxydase, for N fixation and purine metabolism respectively (Reyes *et al*., 2016). It was revealed from the data that application of rhizobium treatments with 100% molybdenum resulted in the best yield for treatment T_5 over the control which suggests that correct bacterization (symbiotic association) along with judicious application of chemical fertilizers and essential cofactors increases growth and yield that might result in increase in net economic return (Table 2). The treatments showed statistically significant protein content for fresh pods of French beans. Highest protein content was noted to be 9.007mg/ml (Fig.1).

Application of molybdenum resulted in yield enhancement through total N accumulation in seeds as well as seed protein content of French bean (Vieira *et al.*, 1998; Somani, 2005; Kandil *et al*., 2013). Increase in available nitrogen was recorded post experimentation for soil which gives an insight of the amount of N being added to the soil per unit (Fig 2).Notable increase in colony forming unit (CFU) was observed that revealed the increase in population of total nitrogen fixers count due to different treatments (Fig 3).

The overall performance of French bean in Coastal Saline Zone of West Bengal was satisfactory. All the combinations of rhizobium, nitrogen and molybdenum had good influence on growth and yield attributes and pod yield of French bean in comparison to the control as revealed from different data output. Microbes related to nitrogen fixation were abundantly found having a combination of Rhizobium V_1B/bean-15+ Nitrogen 50 % + Molybdenum 100 %. Available N after the harvest was high in treatment with a combination of Rhizobium V_1B/bean-15 + Nitrogen 25 % + Molybdenum50 %. Both the treatments may be advocated to the farmers of Coastal Saline Zone of West Bengal for productivity enhancement and maintaining soil sustainability.

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How to cite this article:

Subhankar Biswas, Anannya Banerjee, Pinaki Acharyya and Nirmalya Chakraborty. 2020. Response of French Bean (*Phaseolus vulgaris* L. cv. Arka Arjun) to Rhizobium Inoculation under Varied Levels of Nitrogen and Molybdenum. *Int.J.Curr.Microbiol.App.Sci.* 9(03): 2759-2767. doi: https://doi.org/10.20546/ijcmas.2020.903.316