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Response of *Rhizobium* inoculants and nitrogen application on productivity, uptake of greengram and microbial status of soil

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Abstract
The field experiment comprising four *Rhizobium* inoculation treatments *viz.*, no inoculation, inoculation with *Rhizobium* strains TAL-169, M-10 and AKR-1 respectively in main plots along with three nitrogen nutrition treatments *viz.*, no N application, application of 20 kg N/ha at basal and top-dressing at 30 DAS, respectively in sub-plots, designed in split-plot, was conducted during 2017 and 2018 at the Instructional Farm, Jaguli of Bidhan Chandra Krishi Viswavidyalaya. *Rhizobium* inoculation and time of N application significantly influenced the number of nodules/plant, nodule dry weight/plant and leghaemoglobin content in greengram (cv. B-1). Among the different *Rhizobium* strains C_2, *i.e.*, M-10 strain proved to be efficient in improving the nodule characters and ultimately seed yield of greengram. Inoculation with M-10 *Rhizobium* strain coupled with basal application of 20 kg N/ha increased seed yield of greengram to the extent of 56.6% as compared to no *Rhizobium* inoculation along with no N application. N, P and K uptake by the crop was improved when the seed was inoculated with M-10 strain. *Rhizobium* inoculation also improved the soil health by increasing the population of total bacteria and non-symbiotic N fixing bacteria in soil after harvest of the crop.

Keywords: Nitrogen application, *Rhizobium* inoculation, greengram, productivity

Introduction
Among the grain legumes, mungbean, *i.e.*, greengram (*Vigna radiata* L. Wilczek) is an excellent source of high quality protein. It contains about 25% protein. Mungbean is traditionally a rainy season crop in most of the states of this country, which fails to compete with the high yielding and hybrids of non-legumes produced in this season. The crop is largely grown on marginal and submarginal lands of semi arid and arid tropics which are characterized by low fertility status and moisture stress (Ali and Mishra, 2001) [1]. Leguminous crops (Fabaceae) are known to form symbiotic relationships with soil bacteria, commonly called as root-nodule bacteria or *Rhizobia*. The most important feature of this symbiosis is fixation of atmospheric nitrogen by the bacteria, located inside root nodules, for the benefit of their host plants (Franche *et al*, 2009) [4]. *Rhizobium* inhabiting in its root nodules is efficient source of plant nutrient. *Rhizobia* are ubiquitous soil microorganisms but the diversity and density of soil populations of these bacteria depend on many different factors such as: soil properties, crop rotation, agricultural practices and also to the great extent on the presence of wild species of leguminous plants in a given area. As in the new alluvial zone of West Bengal, generally highly intensive crop cultivation is followed with few areas of green gram cultivation, the native green gram *Rhizobia* population in soil is very low. Inoculation of legume seeds with commercial inoculants containing preselected strains of root-nodule bacteria is a common agriculture practice, which helps to ensure an effective symbiosis, particularly when natural soil populations of these bacteria are deficient, ineffective or only partially effective (Roberts *et al*, 2017) [9]. Response of legumes to inoculation is determined by a variety of factors such as the presence or absence of indigenous *Rhizobia* populations in soil, soil physicochemical constrains, soil nitrogen availability and climatic conditions (Deaker *et al*, 2004) [3]. Effect of nitrogen application on yield of pulse crop was reported by many research workers (Thies *et al*, 2004) [11]. Keeping these two considerations in mind the present investigation was designed to study the effect of different time of nitrogen application and comparative efficiency of different *Rhizobium* strains on productivity, uptake of greengram and microbial status of soil after harvest of the crop.

Materials and Methods

A field experiment was carried out at the Jaguli Instructional farm, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal during the summer season of 2017 and 2018 in the upland conditions. The soil was sandy loam with organic carbon content 0.58%, total nitrogen 0.059%, available P_2O_5 @ 60 kg/ha and available K_2O @ 250 kg/ha. The experiment was laid out in split plot design with 4 main plot treatments of *Rhizobium* cultures (Co: no *Rhizobium* inoculation, C_1: Inoculation with *Rhizobium* strain TAL-169, C_2: inoculation with *Rhizobium* strain M-10, C_3: Inoculation with *Rhizobium* strain AKR-1), and 3 sub plot treatments regarding N application *viz*., no N application (N_0), basal application of nitrogen @ 20 kg N/ha (N_1) and top dressing of 20 kg N/ha at 30 days after sowing (N_2). The greengram variety B-1 was taken for the experiment and sown at spacing of 30 cm x 10 cm with uniform basal application of phosphorus and potassium 60 and 30 kg/ha respectively. *Rhizobium* cultures (M-10, TAL-169 and AKR-1 strain) were mixed in jaggary solution (50 g of jaggery in 500ml of water) and made into slurry. Then pre-soaked seeds (3-4 hours in water) were mixed separately and thoroughly with slurry, dried in shade. Number of nodules per plant and nodule dry weight was taken from ten plants of each plot where as leg- haemoglobin content was measured from the fresh nodules of 5 plants in each plot. Nitrogen, phosphorus and potassium uptake was estimated by modified macro and micro kjeldahl method, Olsen's method and flame photometric method (after tri-acid digestion), respectively (Jensen, 1930) [6]. Total bacteria count was done by using Thornton's agar medium (Thornton, 1922) [12] and counting of aerobic non-symbolic nitrogen fixing bacteria was performed by using Jenson's agar medium (Jackson, 1967) [5].

Results and Discussion

Experimental results revealed that there were significant differences in nodules count due to different *Rhizobium* strains and time of N application (Table 1). Highest number of nodules per plant at 45 DAS was found in the crop treated with M-10 strain (C_2), whereas among the nitrogen application treatment, highest number of nodules/plant was recorded in crop received with basal application of 20 Kg N/ha. In case of nodule dry weight/plant, similar trend was observed. *Rhizobium* inoculation with different strains increased the population of bacteria in host cells which stimulated the nodulation and accompanying nodule growth. Maximum nodule dry weight/plant was recorded when the seed inoculation was done with M-10 strain. Applications of N at basal produced highest nodule dry weight/plant. This might be due to the fact that basal application of N helped in initial root growth which have formed larger domain for bacteriods in host cell to fix N and stimulate nodule growth. The results are in line with the findings of Kozieł *et al*, 2013 [7].

Table 1: Effect of *Rhizobium* inoculation and time of N application on nodule, seed yield, uptake of greengram and change in microbial status of soil (Pooled data of 2017 and 2018)

Treatments	Number of Nodules/ plant at 45 DAS	Nodule dry weight / plant (g/plant) at 45 DAS	Leg haemo-globin content (Mg/g) of fresh nodule) at 45 DAS	Seed yield (Kg/ha)	Uptake (Kg/ha)			Change in Miocrobial status of soil	
					N	P	K	Total bacteria in soil after harvest (CFU × 10^5/g dry soil)	Non-symbiotic N fixing bacteria in soil after harvest (CFU × 10^5/g dry soil)
Main plot: *Rhizobium* Culture									
C_0: (No *Rhizobium* inoculation)	41.86	0.040	117.03	663	154.57	22.93	74.89	74.87	37.96
C_1: (Inoculation with TAL-169 strain)	47.34	0.056	190.88	720	165.18	25.94	84.57	68.07	34.45
C_2: (Inoculation with M-10 strain)	49.47	0.062	202.98	807	173.85	29.16	93.26	88.23	41.13
C_3: (Inoculation with AKR-1 strain)	46.38	0.052	185.42	709	160.41	25.74	80.91	80.34	38.73
CD at 5%	0.76	0.001	10.55	15.90	4.42	0.81	3.17	2.24	0.96
Sub-plot: Time of Nitrogen Application									
N_0: (No nitrogen Application)	43.90	0.046	177.72	635	149.65	22.36	72.08	80.81	38.57
N_1: (Basal application of 20 Kg N/ha)	48.64	0.058	198.36	800	178.44	29.74	94.18	76.76	37.93
N_2: (Top dressing of 20 Kg N/ha)	46.26	0.054	189.97	739	162.42	25.73	83.96	76.06	37.70
CD at 5%	0.99	0.001	15.02	15.30	7.27	1.93	7.31	3.00	N.S.

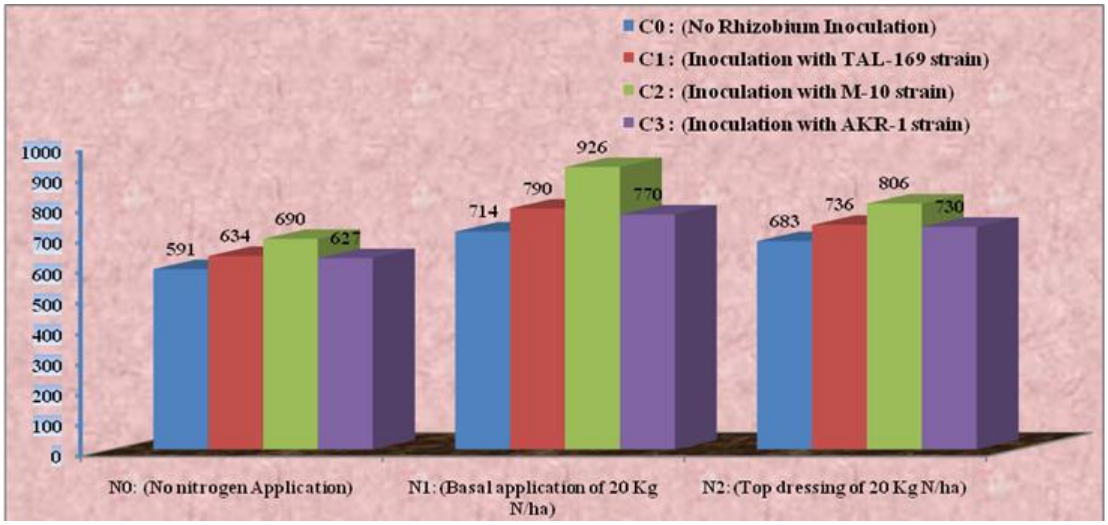

Fig 1: Interaction effect of *Rhizobium* inoculation and time of N application on seed yield of greengram (Pooled Data of 2017 and 2018)

Inoculation by *Rhizobium* increased the secretion of leghaemoglobin from host-cells after nodules were formed from infection thread. So, there was significant increase in the leghaemoglobin content of the fresh nodules due to *Rhizobium* inoculation of seeds over no inoculation. Among the inoculation treatments, M-10 strain produced significantly maximum leghaemoglobin content of fresh nodules where as application of 20Kg N/ha showed highest leghaemoglobin content compare to other nitrogen application treatments.

Seed inoculation stimulated nodulation and encouraged vegetative growth which ultimately reflected on seed yield. Among the different strain of *Rhizobium*, M-10 observed as most efficient. In case of time of nitrogen application, starter dose of 20 Kg N/ha was most suitable for improvement of green gram seed yield (Table 1). Interaction effect of these two was found to be significant (Table 2) for seed yield of greengram. Inoculation significantly increased seed yield over uninoculated control plots of both with or without application of N at different time. The results were confirmed by Kundu *et al.* (2013) [8]. *Rhizobium* inoculation with M-10 strain coupled with basal application of 20 Kg N/ha gave the maximum seed yield as compared to other treatments under consideration. Increased nodulation by *Rhizobium* inoculation boosted the root growth of plants resulting in higher uptake of nitrogen, phosphorous and potassium over no inoculation. However, maximum nitrogen, phosphorous and potassium uptake (173.85, 29.16 and 93.26 kg/ha, respectively) was found in case of M-10 *Rhizobium* strain (C_2) inoculated plants. Similar results were obtained by Singh and Tarafdar (2001) [10]. Time of N application significantly influenced the nitrogen, phosphorous and potassium uptake by plants. Basal application of 20 Kg N/ha fetched significantly highest Nitrogen, Phosphorous and Potassium (178.44, 29.74 and 94.18 Kg/ha, respectively) uptake by plants. Basal application of N showed better result which might be due to the fact that it supplied the initial N requirement for boosting up the early vegetative growth of the plant when nodulation had not started which prompted for higher N uptake by crop whereas, boosting up the early root growth and sustaining mobility of nodule habitating bacteria for longer period might be the possible reason of higher P uptake in basal application of N to plants. The result corroborates the findings of Choudhary *et al*, 2013 [2].

Perusal of results revealed that the TAL-169 (C_1) strain of *Rhizobium* resulted in detrimental influence on the population of total bacteria and non-symbiotic Nitrogen fixing bacteria in soil as compared to those of uninoculated plots. On the other hand, M-10 (C_2) and AKR-1 (C_3) strains of *Rhizobium* resulted in a significant enhancement in proliferation of non-symbiotic N fixer and increased total bacteria population in soil over uninoculated ones. Application of N whether as topdressing or as basal resulted in significant detrimental influence on population of total bacteria in the rhizosphere of greengram. Improvement in population of non-symbiotic N-fixing bacteria in soil after harvest of the crop was recorded when no nitrogen fertilization was done. But the effect was non-significant.

Seed inoculation with M-10 *Rhizobium* strain coupled with basal application of 20 Kg N/ha proved to be better in enhancing the productivity and positive change in bacterial population of soil.

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