JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2017. Vol. **11**(3), p. 1549-1557

Endophytic Plant Growth Promoting Bacteria and Yeast from Mahua flower enhance Growth, Yield and Nutrient Uptake in Greengram

P. Patel[1], H. Desai[2], R. Krishnamurthy[1]* and A. Shah[3]

[1]C. G. Bhakta Institute of Biotechnology, Uka Tarsadia University, Gujarat, India.
[2]Main Cotton Research Station, NAU, Gujarat, India.
[3]Prasad Biotech, Gundlav, Valsad, Gujarat, India.

http://dx.doi.org/10.22207/JPAM.11.3.40

(Received: 10 July 2017; accepted: 25 August 2017)

Among the collection of non-pathogenic endophytes isolated from the flower of mahua, one bacteria (*Bacillus amyloliquefaciens*) and two yeasts (*Rhodotorula* sp. and *Pichia kudriavzevii*) were selected based on their ability to promote growth of greengram. The response of greengram for the growth, yield and nutrient uptake with the microbial inoculation of these three isolates was analyzed individually and in combination under two different field conditions. The experiment was laid out in randomized block design which revealed that single inoculants of both the yeast isolates with biocompost application found superior than recommended dose of chemical fertilizer. Further, the single inoculation of *P. kudriavzevii* and *Rhodotorula* sp. application recorded 31.80 % and 26.93 % more economical and 15.02 % and 12.49 % higher stover yield, respectively than sole biocompost application. The yeast isolates, *P. kudriavzevii* and *Rhodotorula* sp. also recorded 1.31 % and 0.15 % increase in harvest index compared to chemical fertilizer and 10.30 % and 9.04 % increase than sole biocompost application, respectively. Data showed that combinations of the bacteria and yeast inoculums had no additional effects with respect to their single inoculation, but showed superior performance compared to uninoculated controls. This study indicates that a single microbial formulation significantly increases the plant growth and yield in comparison with dual and triple inoculums. The single inoculam of *P. kudriavzevii* showed significantly higher results than in comparison with *B. amyloliquefaciens* and *Rhodotorula* sp. Therefore *P. kudriavzevii* can be a good alternative for chemical fertilizer under South Gujarat region especially for greengram cultivation.

Keywords: Greengram, *Bacillus amyloliquefaciens*, *Rhodotorula* sp., *Pichia kudriavzevii*.

In order to feed ever growing the population, countries like India have to increase the agriculture productivity [1]. Modern agriculture is depending heavily on chemical applications but, environment and sustainable agriculture both are at a risk due to overuse of chemicals; therefore the exploration of effective microbes having plant growth promoting (PGP) activities is required. Flowers of mahua (*Madhuca longifolia*) are traditionally used as bioenhancer as well as a soil amendment to improve soil health [2, 3]. Being a rich source of sugar, flower can act as the outstanding source of endophyte habitat. Therefore, isolation and screening of useful endophytic microbes having PGP properties within this flower were performed and investigated for their PGP effectiveness on non-host plants[4].

Endophytic microbes are believed to elicit plant growth promotion in one of two ways: either indirectly by helping plants acquire nutrients, e.g. via nitrogen fixation, phosphate solubilisation or iron chelation, by preventing pathogen infections via

* To whom all correspondence should be addressed.
Tel.: +91 9825349279;
E-mail: krishnashanti@gmail.com

antifungal or antibacterial agents, by outcompeting pathogens for nutrients by siderophore production, or by establishing the plant's systemic resistance; or directly by producing phytohormones such as auxin or cytokinin [5]. In addition to these PGP traits, endophytic microbes must also be compatible with host plants and able to colonize the tissues of the host plants without being recognized as pathogens [6]. A particular microorganism may affect plant growth and development using one or more of these mechanisms and may use different ones at various times during the lifecycle of the plant. While the growth promotion mechanisms of plant-microbe interactions have produced a large amount of knowledge, it remains unclear how consistently endophytes elicit responses in non-host plant species. Therefore, to exploit application of such PGP microbes on non-host crop, the present study was initiated with greengram (*Vigna radiata* (L.) Wilczek). This crop is popularly known as mung bean and has a major socio-economical importance as being a rich source of nutritious food, animal feed for livestock and raw material for various applications [7] . So, there is an open window to explore endophytes of mahua flowers for their plant growth promoting activities for legumes.

The present study was started with the objective to evaluate the effect of bacterial and yeast endophytes that were previously isolated from mahua flower. Effect of Single and combinatorial inoculation of *Bacillus amyloliquefaciens*, *Rhodotorula* sp. and *Pichia kudriavzevii* was evaluated for growth, yield and nutrient uptake of greengram.

MATERIAL AND METHODS

Experiment site, seed material and land preparation

The field experiment was conducted during two consecutive seasons *viz., kharif*-2015 and summer-2016 at Olpad town located in North West of Surat, India (21.33° N, 72.75° E). The experimental soil was black with clay texture, alkaline pH (8.71) having 0.42 % organic carbon, 207 kg ha^{-1} of available nitrogen (N), 33.20 kg ha^{-1} of available phosphorus (P) and 405 kg ha^{-1} of available potassium (K). The field was prepared by ploughing and the seeds of greengram (cv. Pusa Vishal) drilled by hand in furrows.

Endophyte strains and inoculums preparation

One bacterial strains *Bacillus amyloliquefaciens* MSB1 and two yeast strain *Rhodotorula* sp. MSY1 and *Pichia kudriavzevii* MSY2 were previously isolated from the flower of mahua. All these microorganisms were found to trigger one or more PGP activities. Indole Acetic Acid (IAA) production was the common feature of the three isolated microorganisms. In addition to this, both the strains of yeast showed phosphate solubilization whereas the *B. amyloliquefaciens* is a potent siderophore producer[4]. Further, the above three isolates were found compatible under *in vitro* condition. For inoculums preparation, each isolate was grown in Yeast extract Peptone Dextrose (YPD) medium for 72 hr at 30°C under shaking condition. Each of the three tested strains was grown independently. The final culture was performed in such a way that the cell optical density of bacteria and yeast strain was adjusted to 0.1(at 600 nm) respectively [8].

Field set up to evaluate effect of endophytes

The experiment was planned in Complete Randomize Block Design (CRBD) with 11 treatments and three replications with the plot size of 2.7 × 3.0 m and crop spacing of 0.45 × 0.15 m during two consecutive seasons *i.e, kharif*-2015 and summer-2016. There were 11 treatments comprised of (T1) control, (T2) 100 % RDF (20-40-00 NPK kg ha^{-1}) was supplemented with urea and single super phosphate respectively, (T3) Biocompost at the rate of 2 t ha^{-1}, (T4) T3+MSB1, (T5) T3+MSY1, (T6) T3+MSY2, (T7) T3+MSB1+MSY1, (T8) T3+MSB1+MSY2, (T9) T3+MSB1+MSY1+MSY2, (T10) T3+MSY1+MSY2 and (T11) T3+flower of mahua at rate of 12.5 kg ha^{-1}. All three isolates were applied in soil at the rate of 3 L ha^{-1} with 10^8 colony forming unit mL^{-1} as per the treatment detail.

Three plants were randomly uprooted at 20 and 40 days after sawing, and at harvest to measure shoot length, root length, number of leaves, dry weight of shoot and root. The number of nodules was recorded at 40 days after showing. Yield attributing parameters viz., number of pod per plant, pod weight per plant, length of pod, and seed yield per plant were recorded at harvest from each plot. Economical grain yield, biological yield and stover yield of greengram were also recorded

at harvest and converted on the hectare basis. The harvest index was calculated using formula;
$$(Economical\ grain\ yield \div Biological\ yield) \times 100$$

Mature dried seed and stover sample were subjected to biochemical estimation of N, P and K. Nitrogen estimation were performed as per Kjeldahl method [9]. Phosphorus was estimated by the colorimetric method where the colour of the treated sample reflected the concentration of phosphorus [10]. The potassium was estimated by wet digestion, inductively coupled plasma atomic emission spectrometry [11]. Nutrient uptake (kg ha^{-1}) was estimated by the formula;
$$[(Nutrient\ content\ in\ grain \times grain\ yield) \div 100 + (Nutrient\ content\ in\ stover \times stover\ yield) \div 100]$$

Statistical analysis

Statistical analysis of data for various characters studied in the present investigation was evaluated on the pooled basis. Data analysed using analysis of variance (ANOVA) following complete randomized block design [12]. Data of the effects of treatments were prepared with the standard error of mean and critical different (CD) at 5% probability levels. The coefficient of variance (CV) was calculated and given in the respective tables.

RESULTS

The experiment was performed in CRBD with 11 treatments and three replications. Endophytes from mahua flower were explored as effective inoculums in greengram. Pooled analysis over seasons revealed that the interaction among seasons and treatments was not found significant, which revealed the consistent performance of different treatments during both the seasons. Summer was found more congenial season for greengram cultivation in South Gujarat than *kharif*. Two seasons of trials under different field conditions showed that all three test isolates significantly affected many parameter investigated in comparison to the sole application of biocompost [Table: 1]. The shoot length was recorded maximum in a single application of yeast strain *P. kudriavzevii* followed by *Rhodotorula* sp. On the other end, single inoculation with bacterial isolate *B. amyloliquefaciens* as well as dual yeast inoculation (T10) was found equally effective as chemical treatment. Among all the treatments, the performance of the greengram plants was better with single yeast inoculations in comparison to combined inoculation. Root length was also showed more or less similar performance as shoot length but at harvest, all the treatments having test isolates augmented the higher root length compared to the sole application of chemical, biocompost and control. In general, increased numbers of trifoliate leaves per plant were observed in all treatments over control. However, the numbers of leaves were found higher in both single yeast inoculations. Data presented in [Table 2] showed that dry weight of shoot and root were also found maximum with a single application of yeasts compare to dual and triple inoculation. Regarding numbers of the nodule, the highest nodule was observed with single inoculation of *P. kudriavzevii* and *Rhodotorula* sp. compare to single bacterial inoculation as well as all other combined inoculation. In general, all treatments having test isolate either single or in combination had a significantly increased number of nodule compare to chemical treatment and control.

At harvest similar responses to various treatments was recorded in terms of yield and yield attributing parameters i.e. the number of pod, pod weight and seed yield per plant as well as length of pod [Table 3]. Greater seed yield per plant was observed with single application of *P. kudriavzevii* (19.44 g) which was found comparable to another single as well as dual application (16.91 to 19.24 g). The single application of both yeasts, combined treatments containing yeast had no further stimulatory effect on yield. It was found that greengram plants exerted high grain yield response with both yeast isolates viz. *Rhodotorula* sp. (26.93%) and *P. kudriavzevii* (31.80%) than sole biocompost application. The next effective treatment was the single application with *B. amyloliquefaciens* which was comparable to chemical treatment. The lowest grain yield was recorded in the control treatment. The biological yield was also behaving similarly to the grain yield and exhibited relatively high values with single application of yeast *P. Kudriavzevii* and *Rhodotorula* sp., which was followed by chemical treatment. Additionally, stover yield also followed the similar pattern to above both economic and biological yield. The highest stover yield was again found with the single application of two yeasts isolates *Rhodotorula* sp. (12.49%) and *P. kudriavzevii* (15.02%) over sole application of biocompost. The harvest index did not exhibit much influenced by different microbial inoculation as there was no significant difference among harvest index; however, a comparatively high value was obtained using *P. kudriavzevii* alone (27.94%). This increase was found 10.30% higher than the sole application of biocompost and 1.31% higher for chemical treatment followed by *Rhodotorula* sp. inoculation, which were 9.04% higher than biocompost and 0.15% to RDF. Yeast strain *P. kudriavzevii* and *Rhodotorula* sp. were achieved the highest 1000 seed weight value compare to all other treatment.

Plant nutrient element N, P and K in grain and stover as well as their uptake were presented in [Table: 4]. N content in grain was found significant, but P and K content were not significant among different treatments. The highest N content in grain was found for *P. kudriavzevii* (4.22 %) followed by *Rhodotorula* sp and the lowest were found in control. N and K content in straw were non-significant, whereas P content was found significant between treatments and highest P content (0.19 %) was observed in the single application with *Rhodotorula* sp. Nutrient uptake was estimated and the highest nitrogen uptake was observed with *P. kudriavzevii* alone which was found equally effective with a single application of *Rhodotorula* sp. and chemical treatment. A similar trend of significance was observed for P and K uptake where all of the above treatments recorded significantly superior to the other treatments.

In the present study, bacterial isolate *B. amyloliquefaciens* as well as yeast isolates *Rhodotorula* sp. and *P. kudriavzevii* were found effective in their individual inoculation on growth, yield and nutrient uptake of greengram. However, the dual & triple inoculations were also found more effective than control treatment but remained inferior to RDF. Here, it was found that T11 was poorer to all other treatments having PGP isolates. This might be due to the inadequate density of isolates within mahua flower, a natural source which harbour test isolates. Moreover, the significant increase in all parameters was observed when different PGP isolates were applied

Table 1. Effect on plant growth components under single, dual and triple inoculations with test isolates on greengram (cv. Pusa Vishal)

Treatments	Shoot height (cm)			Root length (cm)			Number of leaves		
	20 DAS	40 DAS	Harvest	20 DAS	40 DAS	Harvest	20 DAS	40 DAS	Harvest
T1	12.54	26.68	33.16	20.67	26.82	29.09	5.67	8.78	10.72
T2	16.74	35.77	43.23	23.27	32.33	34.39	7.00	12.72	15.44
T3	15.16	30.88	37.37	22.59	29.16	32.76	6.28	11.50	12.94
T4	16.31	36.55	41.07	25.14	32.07	36.25	7.00	14.00	15.22
T5	18.42	37.93	44.17	25.62	34.84	37.80	7.22	14.83	16.94
T6	17.34	39.08	45.25	25.88	36.38	38.52	7.67	14.83	17.00
T7	15.63	34.78	39.74	23.19	32.81	36.23	6.33	13.22	14.89
T8	15.23	34.95	40.21	24.82	32.32	37.37	6.83	13.11	15.33
T9	15.68	34.51	39.65	22.82	33.92	37.49	6.17	12.22	13.89
T10	15.29	36.19	41.19	23.50	34.99	36.97	6.28	13.00	14.00
T11	15.66	33.71	39.74	23.43	30.58	33.79	6.22	12.33	13.72
GM	15.82	34.64	40.44	23.72	32.38	35.51	6.61	12.78	14.56
Seasons									
SEM	0.39	0.42	0.43	0.44	0.39	0.35	0.15	0.26	0.20
CD at 5%	1.13	1.19	1.24	1.26	1.10	NS	0.44	0.74	0.71
Treatments									
SEM	0.92	0.98	1.02	1.04	0.91	0.82	0.36	0.60	0.58
CD at 5%	2.64	2.79	2.90	2.96	2.59	2.34	1.03	1.72	1.66
CV %	14.29	6.90	6.15	10.69	6.85	5.65	13.41	11.56	9.79
Interaction									
SEM	1.31	1.38	1.44	1.46	1.28	1.16	0.51	0.85	0.82
CD at 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS

Data presented was pooled over two seasons (kharif 2015 and summer 2016) at 5% level of probability.

as inoculums. This could be possible due to their effective sufficient viable count to enhance rapid colonization in the rhizosphere.

DISCUSSION

Endophytes are ubiquitous in nature. However, the diversity of endophytes has been studied intensively for many plants but not for mahua [13]. Therefore, in this study, endophytes from mahua flower as a novel natural resource were explored for their potential in the field of agriculture. Here, the aim behind screening was to search out effective inoculums consist of microbes with PGP properties so that can be used as an alternative to chemical fertilizer. Since, centuries green gram is widely cultivated in India during two different seasons *viz*, *kharif* and summer due to the short duration and major prospective pulse crop. Therefore, the field study on greengram as test crop should be conducted to investigate the influence of endophytic isolates of mahua flower.

Two seasons of field trials showed that single, dual and triple inoculation with *B. amyloliquefaciens*, *Rhodotorula* sp. and *P. kudriavzevii* significantly enhanced growth, nodulation, seed yield, and content of plant nutrient element as well as their uptake by greengram plant. The enhanced shoot and root length of greengram might be due to the ability of isolates to produce IAA which aid in increasing the plant growth [14, 15]. It was noticed that the entire three consortium member does not contribute equally to the growth of greengram, as all isolates differed in PGP attributes, like IAA, siderophore production, and phosphate solubilization. Therefore, in T6; MSY2 being the highest producer of IAA and P-solubilizer showed better plant growth response followed by T5 (MSY1) than other treatments. Against untreated control, numbers of leaves per plant were found significantly among different treatments. These leaves correspond to added light interception, which results into addition biomass and higher plant productivity. Therefore,

Table 2. Effect of test inoculums on dry weight of plant and nodulation

Treatments	Shoot dry weight (g)			Root dry weight (g)			Number of nodules
	20 DAS	40 DAS	Harvest	20 DAS	40 DAS	Harvest	40 DAS
T1	3.28	10.83	34.17	0.27	0.88	1.31	66.78
T2	4.65	18.09	62.58	0.61	1.28	1.98	96.50
T3	4.16	15.64	44.96	0.41	1.08	1.55	95.61
T4	4.26	18.83	60.57	0.55	1.35	2.17	104.39
T5	4.42	20.21	64.81	0.81	1.62	2.28	113.83
T6	4.42	21.27	64.59	0.84	1.74	2.32	117.94
T7	4.34	17.43	55.58	0.42	1.32	1.78	105.50
T8	4.32	18.08	58.42	0.45	1.37	2.03	99.78
T9	4.51	16.18	49.84	0.55	1.48	1.99	103.94
T10	4.20	18.64	55.07	0.60	1.32	2.07	102.00
T11	4.32	15.79	47.67	0.41	1.14	1.75	95.89
GM	4.26	17.36	54.39	0.54	1.32	1.93	100.20
Seasons							
SEM	0.07	0.42	1.23	0.01	0.03	0.05	1.68
CD at 5%	0.19	1.19	3.50	0.02	0.10	0.13	NS
Treatments							
SEM	0.16	0.98	2.88	0.02	0.08	0.11	3.94
CD at 5%	0.45	2.79	8.22	0.05	0.23	0.31	11.25
CV %	8.95	13.76	12.95	8.63	14.86	13.72	9.45
Interaction							
SEM	0.22	1.38	4.07	0.03	0.114	0.15	5.57
CD at 5%	NS	NS	NS	NS	NS	NS	NS

Data presented was pooled over two seasons (kharif 2015 and summer 2016) at 5% level of probability.

plant dry matter was also affected by inoculation with different PGP isolates. In addition to these, improvement of the plant biomass was increased due to nutrients absorption, which leads to high rate of translocation and ultimately more dry matter accumulation. The similar observation was also made on green gram by previous researchers with various bioinoculants [16].

Numbers of nodules per plant can also be affected by microbial inoculation. A range of evidence has been reported by many investigators on PGP microbes stimulation of nodulation, as well as the creation of more infection sites on plant root [17]. The isolates having better P-solubilization ability showed better nodulation, when compared to the uninoculated greengram, which showed the least number of nodules. These results suggest that the presence of native rhizobial of greengram in the experimental soil is of the inadequate number. Subsequently, IAA production enhanced the root length and provides larger surface area for more bacteroids attachment, leading to more nodulation. All these treatments had a significant number of nodule compare to untreated and RDF treatments. The similar observation was made by PGP non *Rhizobium* bacteria on greengram in previous studies [18]. It is well understood that available P is needed for the nodule infection process by *Rhizobium*. The supply of essential inorganic P to bacteroids in relation to the symbiosome may affect the effectiveness of nitrogen fixation and nodulation [19]. This suggests that growth and nodulation respond to P supply. In our study, both yeast strains possess the P-solubilizing ability which may result in higher nodulation in greengram. Treatments having isolates exerted good plant growth by means of higher stover as well as grain yields under the field condition over the control. It was observed that stover yield was increased with the increase in vegetative growth. The reason might be the effective utilization of nutrient absorption through extended root system which resulted in

Table 3. Influence on yield and its components under different inoculations with test isolates on greengram (cv. Pusa Vishal)

Treatments	Number of pod	Pod weight /plant (g)	Length of pod (cm)	Seed yield/ plant (g)	Grain yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Stover yield (kg ha^{-1})	Harvest index (%)	1000 seed weight (g)
T1	28.22	18.29	7.84	11.51	1006.1	4251.05	3244.95	23.47	41.64
T2	43.22	32.59	8.45	18.59	1519.62	5471.97	3952.35	27.58	43.9
T3	32.78	22.38	8.19	14.42	1209.34	4755.23	3545.9	25.33	42.41
T4	43.67	30.13	8.75	17.18	1393.44	5099.59	3706.14	27.18	43.49
T5	45.67	33.02	9.03	19.24	1535.03	5523.71	3988.67	27.62	44.44
T6	47.33	35.11	9.05	19.44	1593.87	5672.51	4078.64	27.94	44.45
T7	39.5	31.23	8.51	16.91	1284.82	4914.07	3629.25	25.97	43.47
T8	43.83	32.26	8.55	18.72	1266.48	4889.95	3623.47	25.76	44.04
T9	40.06	26.55	8.7	15.84	1323.44	5058.31	3734.88	26.02	43.26
T10	38.17	27.06	8.55	16.47	1358.49	5095.2	3736.71	26.52	43.39
T11	35.94	24.06	8.33	15.03	1264.89	4963.5	3698.6	25.38	42.52
GM	39.85	28.43	8.54	16.67	1341.41	5063.19	3721.78	26.25	43.36
Seasons									
SEM	0.9	0.67	0.04	0.41	24.74	48.75	38.44	0.38	0.08
CD at 5%	2.56	1.91	0.11	1.17	70.7	139.35	109.87	1.07	0.23
Treatments									
SEM	2.1	1.57	0.09	0.96	58.01	114.34	90.15	0.88	0.19
CD at 5%	6	4.47	0.26	2.74	165.82	326.8	257.66	2.51	0.53
CV %	12.91	13.49	2.64	14.07	10.59	5.53	5.93	8.2	1.06
Interaction									
SEM	2.97	2.21	0.13	1.35	82.04	161.69	127.49	1.24	0.26
CD at 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS

Data presented was pooled over two seasons (kharif 2015 and summer 2016) at 5% level of probability.

proliferated shoot development. The highest grain yield was observed with T6; MSY2 followed by T5; MSY1. These suggested cumulative effects of both yeasts isolates between growth and yield attributes. Not only IAA, other PGP activities of microbes such as phosphate solubilization also stimulate the absorption efficiency of nutrients and photosynthesis metabolism that promote the plant growth and thereby yield [20]. Both the yeast isolate *Rhodotorula* sp. and *P. kudriavzevii* contributed more to enhance yield by way of increasing the number of pod and seed per plant in comparison to bacterial isolate *B. amyloliquefaciens* and all other co-inoculation and RDF treatment. The increase in harvest index again reconfirmed the positive correlation between grain yield and stover yield.

Though there was no known nitrogen-fixing strains of isolates used, the significant increased in seed N content was found which supported the hypothesis that biological nitrogen fixation by the *Rhizobium* and other root associative microbes could be responsible for the observed higher N uptake of inoculated plant [21]. Furthermore, the increased P content in straw on the application of both yeast isolates suggested their establishment in the greengram rhizosphere. Both these yeast isolates have a P solubilization property which may lead higher uptakes of P and reflected in better yield attributes. It is a well-known that phosphate solubilising yeast releases an organic acid that increases the availability of P in their soluble form and ultimately enhanced plant growth development [22]. The increased growth and yield of legume crops following inoculation with phosphate solubilising microorganisms have been also reported previously [20]. Due to this, availability of nutrients raised and which were resulted in greater uptake by the plants and ultimately increases their concentration. Increased N, P and K content of greengram given the additional evidence to the finding of the previous study [23]. The resultant improvement of nutrient uptake due to combinatorial activities of the isolates and plant were also reported earlier [24].

In the present study, all three isolates *B. amyloliquefaciens*, *Rhodotorula* sp. and *P. kudriavzevii* were found effective in their single inoculation. As per the hypothesis of the present work dual inoculation with IAA and siderophore

Table 4. Effect of various microbial inoculants on nutrient contents and their uptake by greengram (cv. Pusa vishal) at harvest.

Treatments	Nutrient content						Nutrient uptake		
	Nitrogen in plant (%)	Nitrogen in seeds (%)	Phosphorus in plant (%)	Phosphorus in seeds (%)	Potash in plant (%)	Potash in seeds (%)	Nitrogen (kg ha^{-1})	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})
T1	1.67	3.54	0.14	0.26	1.39	1.15	91.42	7.03	55.80
T2	2.10	3.70	0.16	0.37	1.75	1.19	139.44	11.88	86.28
T3	1.76	3.52	0.14	0.30	1.60	1.15	105.39	8.65	69.74
T4	2.17	3.58	0.14	0.32	1.66	1.19	131.90	9.73	76.06
T5	2.57	4.07	0.19	0.34	1.85	1.17	164.91	12.61	91.45
T6	2.48	4.22	0.17	0.35	1.78	1.19	169.80	12.41	90.78
T7	1.80	3.94	0.17	0.29	1.76	1.18	117.36	9.81	79.11
T8	1.83	4.04	0.16	0.34	1.60	1.17	117.52	10.08	72.25
T9	1.82	3.81	0.16	0.33	1.54	1.15	118.50	10.19	72.66
T10	1.83	3.62	0.14	0.34	1.61	1.15	118.24	9.84	75.09
T11	1.93	3.84	0.15	0.33	1.64	1.17	120.04	9.69	74.40
GM	1.99	3.81	0.16	0.32	1.65	1.17	126.77	10.18	76.69
Seasons									
SEM	0.12	0.05	0.00	0.01	0.05	0.01	4.54	0.21	1.87
CD at 5%	NS	NS	NS	NS	0.13	0.02	12.98	0.61	NS
Treatments									
SEM	0.27	0.11	0.01	0.03	0.11	0.02	10.65	0.50	4.38
CD at 5%	NS	0.32	0.02	NS	NS	NS	30.43	1.43	12.51
CV %	33.68	7.10	12.76	20.73	15.97	3.75	20.57	12.06	13.98
Interaction									
SEM	0.39	0.16	0.01	0.04	0.15	0.03	15.06	0.71	6.19
CD at 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS

Data presented was pooled over two seasons (kharif 2015 and summer 2016) at 5% level of probability.

producing *B. amyloliquefaciens* with IAA and P solubilising yeasts *Rhodotorula* sp and/or *P. kudriavzevii* were expected to improve highest plant growth. Other reported studies concluded that the combined inoculation significantly increased growth and yield when compared to their individual inoculation [16, 25]. Contradictory to that, our study revealed that dual and triple inoculation had significantly lower yields than those of single inoculations. This might be due to the nutritional competition among microorganisms and plant. It was previously reported that the rhizosphere competence between plant and native soil flora for carbon sources also limited plant growth [26]. Siderophore is small, high-affinity iron-chelating compounds, secreted by many PGP microorganisms under the stress conditions, which help in plant development. Additionally, siderophore creates competition for irons and other nutrients, which is based on the supposition where nutrients availability may limit the microbial growth and their development on plant surfaces [27]. Bacterial isolate *B. amyloliquefaciens* was the good siderophore producer. An alkaline condition of soil increased the stress which enhanced the siderophore production and caused the nutrition competition for other co-inoculums members, soil inheritance flora as well as for plant. This was the reason for their inferior performance.

CONCLUSION

Colonization of microbes with their hosts could be beneficial or harmful and affect the populations antagonistically. Therefore, it is needed to understand the specific ecological niche for a better outcome. From the study, it can be concluded that isolates viz. *B. amyloliquefaciens*, *Rhodotorula* sp. and *P. kudriavzevii* tested in this study found potent microbial fertilizer as the single applicant to achieve maximum productivity of greengram and have potential to substitute costly NP fertilizer in greengram production.

ACKNOWLEDGEMENT

Authors are grateful to Mr. Dipak Patel, farmer at Olpad village for providing all required facilities for field study and Dr. Jayesh Pastagiya, Dr. Sanjay Jha from Navsari Agricultural University for their continuous guidance and encouragement. Authors also express sincere gratitude to C. G. Bhakta Institute of Biotechnology, Uka Tarsadia University.

REFERENCES

1. Mia, M.B., Shamsuddin, Z. *Rhizobium* as a crop enhancer and biofertilizer for increased cereal production. *Afr. J. Biotechnol.*,2010; **9**(37):6001-9.
2. Pathak, R., Ram, R. Bio-enhancers: A potential tool to improve soil fertility, plant health in organic production of horticultural crops. *Progressive Horticulture.* 2013; **45**(2):237-54.
3. Jha, S., Vaibhav, V., Suneetha, V.A Culinary Mahua (*Madhuca indica*) flower from Bihar, India? A potential in production of Jam, alcohol for pharmacological benefits with fertilizer value. *Int. j. drug dev.*, 2013; **5**(2):362-7.
4. Patel P., Krishnamurthy R., Shah A . *In vitro* evaluation of selective mahua flowers endophytes as a bioinoculant on greengram [*Vigna radiata* (L.)]. *Int. J. Pharma Bio sci.*(in press).
5. Santoyo, G., Moreno-Hagelsieb, G., del Carmen Orozco-Mosqueda, M., Glick, B.R. Plant growth-promoting bacterial endophytes. *Microbiol. Res.* 2016; **183**:92-9.
6. Dudeja, S., Giri, R., Saini, R., Suneja Madan, P., Kothe, E. Interaction of endophytic microbes with legumes. *J. Basic. Microbiol.* 2012; **52**(3):248-60.
7. Singh R., Status paper on pulses. Directorate of pulse development, Bhopal. 2013: 89-90.
8. Kaczorek, E., Bielicka-Daszkiewicz, K., Héberger, K., Kemény, S., Olszanowski, A., Voelkel, A. Best conditions for biodegradation of diesel oil by chemometric tools. *Braz. J. Microbiol.* 2014; **45**(1):117-26.
9. Nelson, D., Sommers, L. Determination of total nitrogen in plant material. *Agron J.* 1973; **65**(1):109-12.
10. Noureddini, H., Malik, M., Byun, J., Ankeny, A. Distribution of phosphorus compounds in corn processing. *Bioresour Technol.* 2009; **100**(2):731-6.
11. Zarcinas, B., Cartwright, B., Spouncer, L. Nitric acid digestion and multi element analysis of plant material by inductively coupled plasma spectrometry. *Commun. Soil Sci. Plant Anal.* 1987; **18**(1):131-46.
12. Gomez, K.A., Gomez, A.A. Statistical procedures for agricultural research: John Wiley & Sons; 1984.

13. Compant, S., Mitter, B., Colli-Mull, J.G., Gangl, H., Sessitsch, A. Endophytes of grapevine flowers, berries, and seeds: identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. *Microb. Ecol.* 2011; **62**(1):188-97.
14. Kamilova, F., Kravchenko, L.V., Shaposhnikov, A.I., Azarova, T., Makarova, N., Lugtenberg, B. Organic acids, sugars, and L-tryptophane in exudates of vegetables growing on stonewool and their effects on activities of rhizosphere bacteria. *Mol. Plant-Microbe Interact.* 2006; **19**(3):250-6.
15. Ali, B., Sabri, A.N., Ljung, K., Hasnain, S. Quantification of indole-3-acetic acid from plant associated Bacillus spp. and their phytostimulatory effect on *Vigna radiata* (L.). *World J. Microbiol. Biotechnol.* 2009; **25**(3):519-26.
16. Vendan, R.T. Studies on the effect of various bioinoculants on the growth and yield of greengram (*Vigna radiata* L.). *Food Legumes*. 2016; **29**(1):43-5.
17. Vessey, J.K., Buss, T.J. *Bacillus cereus* UW85 inoculation effects on growth, nodulation, and N accumulation in grain legumes: controlled-environment studies. *Can. J. Plant Sci.* 2002; **82**(2):282-90.
18. Sahu, G., Sindhu, S. Disease control and plant growth promotion of green Gram by siderophore producing *Pseudomonas* sp. *Res. J.Microbiol.* 2011; **6**(10): 1-15.
19. Yang Y. The effect of phosphorus on nodule formation and function in the *Casuarina-Frankia* symbiosis. *Plant Soil.* 1995; **176**(1):161-9.
20. Zaidi, A., Khan, M.S. Co-inoculation effects of phosphate solubilizing microorganisms and Glomus fasciculatum on green gram-*Bradyrhizobium* symbiosis. *Turk. J. Agric For.* 2006; **30**(3):223-30.
21. Elkoca, E., Kantar, F., Sahin, F. Influence of nitrogen fixing and phosphorus solubilizing bacteria on the nodulation, plant growth, and yield of chickpea. *J. Plant Nutr.* 2007; **31**(1):157-71.
22. Mundra, S., Arora, R., Stobdan, T. Solubilization of insoluble inorganic phosphates by a novel temperature-, pH-, and salt-tolerant yeast, *Rhodotorula* sp. PS4, isolated from seabuckthorn rhizosphere, growing in cold desert of Ladakh, India. *World J. Microbiol. Biotechnol.* 2011; **27**(10):2387-96.
23. Zaidi, A., Khan, M.S., Aamil, M.. Bioassociative effect of rhizospheric microorganisms on growth, yield, and nutrient uptake of greengram. *J. Plant Nutr.* 2004; **27**(4):601-12.
24. Raynaud X., Lata J.C., Leadley, P.W. Soil microbial loop and nutrient uptake by plants: a test using a coupled C:N model of plant–microbial interactions. *Plant Soil.* 2006; **287**(1-2):95-116.
25. Khan, Z., Guelich, G., Phan, H., Redman, R., Doty, S. Bacterial and yeast endophytes from poplar and willow promote growth in crop plants and grasses. ISRN Agronomy. 2012; 2012:1-11.
26. Oliveira, A.d., Urquiaga, S., Döbereiner, J., Baldani, J. The effect of inoculating endophytic N_2-fixing bacteria on micropropagated sugarcane plants. *Plant Soil.* 2002; **242**(2):205-15.
27. Loper, J.E., Buyer, J.S. Siderophores in microbial interactions on plant surfaces. *Mol Plant-Microbe Interact.* 1991; **4**(1):5-13.