INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2004/06–5–837–843
http://www.ijab.org

Comparative Study of the Effects of Biofertilizers on Nodulation and Yield Characteristics of Mung Bean (*Phaseolus vulgaris* L.)

SAEED AHMAD ASAD[1], ASGHARI BANO, MUHAMMAD FAROOQ†, MUHAMMAD ASLAM‡ AND AFTAB AFZAL
Department of Biological Sciences, Quaid-i-Azam University, Islamabad–Pakistan
†*Department of Crop Physiology, University of Agriculture, Faisalabad–38040, Pakistan*
‡*National Agricultural Research Centre, Park Road, Islamabad–Pakistan*
[1]Corresponding author's e-mail: saeedasadrana@hotmail.com

ABSTRACT

The present investigation was aimed at determining the effects of biofertilizers and their pure cultures on phytohormones production, plant growth and yield. Changes in the level of phytohormones were also determined in plant leaves inoculated with biofertilizers and pure cultures used alone or in co-inoculation. In pot experiment, three biofertilizers (Biopower, Biozote & Grasimi Tika) were applied to one group of plants as seed coating treatment. To a second group, broth cultures (10 days after inoculation) of the rhizobial strains were applied to seedlings (7 days after germination). Biopower resulted in maximum IAA and GA content in plant leaves than Biozote and Grasimi Tika. IAA and GA content of Biopower were higher than that of Biozote and Grasimi Tika. Biopower inoculation also resulted in an increase in nodule numbers, root/shoot length, seed weight and yield while, Biozote treatment resulted in yield that was slightly lower than Biopower, but was at par with all other treatments. Among the rhizobial strains, MN-Slow produced maximum IAA and GA in broth culture and minimum IAA and GA were observed in strain CA-18. Co inoculation of rhizobium strains showed better results than applied singly. IAA and GA contents of leaves of treated plants were also recorded to be maximum in co-inoculation treatments.

Key Words: Biofertilizer; Nodulation; Yield; Mung bean; Phytohormone

INTRODUCTION

Mung bean is an important world food crop for providing an inexpensive source of vegetable protein. World production of mungbean is about 1.24 million tones per annum from an estimated total area of 3.21 million hectares (Government of Pakistan, 2003). In Pakistan it is grown on 261.4 thousand ha with the production of 134.4 thousand tones of grain annually (Government of Pakistan, 2003). Mungbean is a sub-tropical, kharif crop, well adapted to semiarid and sub humid zones with annual rainfall between 600-1000 mm requiring an optimum mean temperature 30°C. It grows successfully on sandy loam to clay loam soils, usually grown on low to medium elevations in the tropics as a rain fed crop (Ahmad & Jan, 2002). It is a short duration crop and takes from 65-120 days to mature (Smith & Roberts, 1987).

Biological nitrogen fixation. In legumes, N_2 fixation starts with the formation of root nodules. *Rhizobium* cells invade the root and reside within the cortex cell. Within a week after infection, small nodules are visible with naked eye. As the nodules grow in size, they gradually turn pink to reddish in color, indicating that N_2 fixation has started. Under field conditions, nodulation process of mungbean and other legume crops are adversely affected by various soil and environmental factors such as acidic soil pH (Rengel, 2002), low phosphorous (Mullen, 1988). Uneven distribution of water i.e., drought (Serraj & Sinclair, *1998*) or excessive moisture conditions (Lupwai *et al.,* 1997) can also inhibit nodulation process. The total amount of N-fixed through biological system was estimated to be 100-175 million metric tones per year (Ishizuka, 1992).

Biofertilizers. Biofertilizers may be classified as: Carrier-based inoculants fixing atmospheric nitrogen or by stimulating plant growth through synthesis of growth promoting substances; Blue green algae or *Cynobacteria mycorrhizae*.

Biofertilizers can add 20-200 kg N ha^{-1} (by fixation), liberate growth-promoting substances and increase crop yield by 10-50%. They are cheaper, pollution free, based on renewable energy sources and also improve soil tilth (Motsara *et al.,* 1995*)*.

Biofertilizers manufactured and sold commercially by different research organizations in Pakistan include:

Biozote. Biofertilizer manufactured by Pakistan Agricultural Research Council (PARC) Islamabad. It is composed of living bacteria TAL-169 in the carrier material.

Grasimi tika. Biofertilizer manufactured by Ayub Agricultural Research Institute (AARI), Faisalabad. The Rhizobium strain used is SM-101in the carrier material leaf mold collected from Change Manga forest plantation.

Biopower. Biofertilizer manufactured by National Institute for Biotechnology and Genetic Engineering (NIBGE) Faisalabad consisting of three Rhizobial strains TAL102, MN-Slow and CA-18 in the carrier material pressed mud.

Plant growth hormones. Plant growth hormones are naturally occurring compounds in plants and are involved in several biochemical and physiological processes. Microorganisms like Azospirillum, Alcaligenes, Klebsiella, Enterobacter, Xanthomonas, Pseudomonas, Rhizobium and Bradyrhizobium can synthesize auxins (Martinez *et al.*, 1998), gibberellins (Rasul *et al.*, 1998) in plants as well as in broth culture. Objective of this study was to see the difference in efficiencies of different carrier based biofertilizers (as well as their pure cultures) manufactured and sold commercially by different research organizations in Pakistan. Secondly to evaluate the hormone production capability of different biofertilizers and their pure cultures in vitro and to see the storage effect on phytohormone production by Biofertilizers.

MATERIALS AND METHODS

Plant material and biofertilizers. Seed of Mungbean (*Phaseolus vulgaris* L.) cv. NCM 209 were obtained from the Pulses section of Institute of Field and Horticultural Crops (IFHC) at National Agricultural Research Center (NARC) Islamabad. The experiment was carried out in earthen pots ($24x30cm^2$) in the green house of the Department of Biological Sciences.

Biofertilizers (Biopower, Biozote, Grasimi Tika) and five pure cultures of *Rhizobium* (MN-Slow, TAL-102, CA-18, TAL-169, SM-101) were used alone as well as in combination to inoculate the plants in the experiment. Biopower and three pure cultures of *Rhizobium* (MN-Slow, TAL-102, and CA-18) were obtained from National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad. Grasimi Tika and its pure culture of *Rhizobium* (SM-101) were obtained from Ayub agricultural Research Institute (AARI), Faisalabad. Biozote and its pure culture of *Rhizobium* (TAL-169) were obtained from the National Agricultural Research Centre (NARC), Islamabad.

Treatments. (I). Control (uninoculated) denoted as T_1 (II). Biopower (seeds inoculated with carrier based inocula) denoted as T_2 (III). TAL-102+MN-Slow+CA-18 (seedlings inoculated with broth cultures of *Rhizobium*) denoted as T_3. (IV). TAL-102+MN-Slow denoted as T_4. (V). TAL-102+CA-18denoted as T_5. (VI). MN-Slow+CA-18 denoted as T_6. (VII). MN-Slow denoted as T_7. (VIII). TAL-102 denoted as T_8. (IX). CA-18 denoted as T_9. (X). Biozote (seeds inoculated with carrier-based inocula) denoted as T_{10}. (XI). TAL-169 (seedlings inoculated with broth culture of *Rhizobium*) denoted as T_{11}. (XII). Grasimi Tika (seeds inoculated with carrier based Inocula) denoted as T_{12}. (XIII). SM-101 (seedlings inoculated with broth culture of *Rhizobium*) denoted as T_{13}.

Method of inoculation. (A) seed coating: Seed of mungbean (*Phaseolus vulgaris L.*) were surface sterilized with 0.1% Mercuric Chloride ($HgCl_2$) for 3 min and subsequently washed in distilled water for 6-7 times. These surface sterilized seeds were moistened in sugar solution (48%) before application of inoculum to get a thin, uniform coating of Inoculum on seeds. Inoculated seeds were dried in shade before sowing (Samasegaran *et al.*, 1982).

(B) Inoculation of seedlings. 7-days after germination the seedlings in pots were inoculated with broth cultures of *Rhizobium* (10 days old @ 10 ml /plant) grown in Yeast Mannitol (YM) broth medium in a shaker (revolving at 75 rpm) at 28±2°C and after that preserved in the refrigerator at 4ºC. Number of cells at the time of application was 10^7 in all the strains and conditions and days after inoculation were same for all the strains used in different combinations.

Detection of IAA and GA in Biofertilizers (freshly manufactured and 45 Days after sowing at room temperature. 100 g of each of three biofertilizers were suspended in 100 ml distilled water, centrifuged at 5000 rpm for 20 minutes. Supernatant was adjusted to pH 2.3–3.0 with 1N HCl and partitioned three times with 1/3 volume of ethyl acetate for the extraction of hormones. The aqueous phase was discarded and organic phase was dried down completely using Rotary thin Film Evaporator. Dried sample from the rotary flask was dissolved in 1ml of methanol and analyzed on High Performance Liquid Chromatography (Shimadzu, C-R4A chromatopac, ScL-6B system controller) equipped with variable UV detector and C-18 column ($39 \times 300mm^2$) following the procedure of Lie *et al.*, (1994) for detection of IAA & GA.

Detection of IAA and GA in broth culture (10 day old) of *Rhizobium leguminosarum.* The YM broth (150ml) was inoculated with 5day old starter cultures of *Rhizobium* and placed on a shaker revolving at speed of 75 rpm at 28±2°C. These bacterial cultures were centrifuged at 10,000 rpm for 20 minutes and supernatants were adjusted to pH 2.5-3.0 with 1N HCl**.** Hormones were extracted by partitioning the supernatants with 1/3 volume of ethyl acetate as demonstrated by Tien *et al.* (1979) and analyzed on HPLC as already discussed.

Detection of IAA and GA Content of plant leaves before and after nodulation. Extraction and purification of Indole acetic acid (IAA) and Gibberellic Acid (GA) from plant leaves were made following the procedure of Kettner and Droffling (1995). 2g Fresh leaves from the plants were taken and rinsed in distilled water for 2-3 times to remove the dust etc. These leaves were stored in the refrigerator at 4c then ground in 80% methanol; by using butylated hydroxy toluene (BHT) as antioxidant. The supernatant (after 72 h extractions) was dried down using Rotary Film Evaporator (RFE) and same procedure was adopted for the analysis of sample as stated above.

Number of mature nodules/plant. After 35 days of emergence, plants from three replicates were pulled out, with roots, washed in distilled water and fully mature nodules (pink colored) per plant were counted and average was computed.

Number of Seeds/pod, 100 seed weight and Seed yield. Three pods from each replicate were taken at random and threshed to count the number of seeds in each pod

Table I. Concentration of IAA and GA (mg/kg) in biofertilizers (freshly manufactured As well as 45 DAS)

Treatments	IAA*	GA**	IAA***	GA****
Biopower	5.147 a	1.053 a	46.00 a	0.5880 a
Biozote	3.203 b	0.3667 b	0.840 c	0.1317 b
Grasimi Tika	3.730 b	0.2667 b	18.70 b	0.2663 b

LSD value at 0.05=1.352*, 0.5721**, 10.91***, 0.1548****; Figures not sharing the same letter differ significantly at p 0.05

Table II. Concentration of IAA and GA (mg/L) in broth culture (10 days old) of *Rhizobium eguminosarum*

Treatments	IAA*	GA**
MN-Slow	7.157 a	14.85 a
TAL-102	6.393 a	10.69 b
Ca-18	1.987 c	7.573 c
TAL-169	4.307 b	11.93 b
SM-101	6.657 a	8.190 c

LSD value at 0.05=1.808**, 1.729**; Figures not sharing the same letter differ significantly at p 0.05

Table III. Effects of carrier-based biofertilizers and pure cultures of the strains of *Rhizobium leguminosarum* on the IAA and GA concentration (mg/kg) in mung bean leaves before nodulation (20 DAS)

Treatments	IAA*	GA**
Control (uninoculated)	0.5033 f	0.6667 h
Biopower	11.87 a	6.680 a
TAL-102+MN-slow+Ca-18	9.183 b	3.567 d
TAL-102+MN-Slow	7.820 c	2.607 ef
TAL-102+Ca-18	7.440 cd	1.240 g
MN-Slow + Ca-18	9.917 b	5.437 b
MN-Slow	9.967 b	4.450 c
TAL-102	6.690 d	2.847 e
Ca-18	5.030 e	2.117 f
Biozote	4.643 e	2.140 f
TAL-169	4.483 e	2.130 f
Grasimi tika	9.447 b	6.683 a
SM-101	9.427 b	3.807 d

LSD value at 0.05=0.8188*, 0.5886**; Figures not sharing the same letter differ significantly at p 0.05

separately. Number of seeds was counted and average was worked out. 100 seed weight was calculated from the seed lot of each treatment and then average was computed. At the end yield was recorded from each pot separately and then average was computed.

Physico chemical analysis of soil. Soil sample (25g) was suspended in 25ml-distilled water and stirred for 10 minutes and the pH was recorded with a pH meter following the recommended soil chemical test procedures (1988). Pre sowing analysis of soil showed pH, NO_3N, and P as 6.5, 3.73 ppm and 5.14 ppm, respectively follows the procedure of Mehlich (1984).

RESULTS

In freshly manufactured biofertilizers the concentration of IAA* and GA** was maximum in the Biopower which was significantly higher than that of IAA produced either by Biozote or Grasimi Tika (Table I).The concentration of IAA and GA of freshly manufactured Biozote and Grasimi Tika was about I fold lower than Biopower did not differ significantly from each other.

Data demonstrated that concentration of IAA*** in Biopower under storage was recorded to be maximum and minimum in the Biozote, differing significantly from the concentration of IAA in Grasimi Tika. IAA concentration in the Biopower was about 2 folds higher than IAA concentration in Grasimi Tika and 5 folds higher than IAA concentration in the Biozote.

Concentration of GA**** was found to be significantly higher in Biopower than Grasimi Tika and Biozote for observations made 45 days after storage. GA in the Biopower was recorded 2 folds higher than that of Grasimi Tika however; GA concentration of Biozote was 4 folds less than that of Biopower and 1 fold less than that of Grasimi Tika.

Among the Rhizobial strains (MN-Slow, TAL-102, CA-18) constituting biofertilizer, Biopower maximum IAA was detected in the broth culture of MN-Slow which did not differ significantly with IAA content of TAL-102 but was 6 folds higher than IAA in CA-18 (Table II). IAA concentration detected in SM-101 (strain used in the, Grasimi Tika) was significantly higher than IAA in the broth culture of TAL-169 (strain used in the, Biozote).

GA concentration was also found to be significantly higher in the broth culture of MN-Slow. GA detected in the broth cultures of TAL-102 and TAL-169 was statistically similar.

Results showed that IAA was increased significantly in the leaves of plants inoculated with biofertilizers as well as pure cultures of *Rhizobium* strains as compared to control (Table III). Plants inoculated with Grasimi Tika showed more than 2 folds greater IAA than that produced by the plants inoculated with Biozote although; the value was much less than that of Biopower.

Plants inoculated with pure culture of MN-Slow either used alone or in combination with CA-18 or CA-18+TAL-102 exhibited IAA content significantly lower than that of Biopower but, there did not appear any significant difference in the IAA content of leaves of plants inoculated with any of these cultures alone or in combination. Inoculation with Biozote and its pure culture resulted in the production of IAA statistically similar. Similar pattern for IAA production was observed in the Grasimi Tika and its pure culture.

GA content was found to be maximum in the leaves of plants inoculated with Grasimi Tika which was statistically similar to the GA produced by Biopower, but differed significantly from that produced by Biozote.

Plants inoculated with pure culture of *Rhizobium* strain MN-Slow exhibited GA content significantly lower than it was applied in co-inoculation with CA-18.

The carrier-based inoculum Biozote and its pure culture TAL-169 resulted in the production of similar amount of GA however, *Rhizobium* strain SM-101 produced GA, which were 3 folds less than that produced by Grasimi Tika.

Plants inoculated with Biopower showed maximum IAA than produced by Biozote or Grasimi Tika. Biozote inoculation resulted in minimum IAA production (Table IV). Plants inoculated with *Rhihizobium* strain MN-Slow either alone or in co-inoculation with CA-18 and TAL-102+CA-18 produced IAA which was significantly lower than that produced by Biopower. There was no significant difference in the IAA concentration of the Grasimi Tika and its pure culture and that of Biozote and its pure culture.

GA increased significantly in the leaves of inoculated plants than uninoculated plants (control). Maximum GA was recorded in the plants inoculated with Biopower, which were 3 folds greater than GA produced in plants treated with Biozote.

Plants inoculated with *Rhizobium* strain MN-Slow either used alone as well as in combination with TAL-102 +CA-18 produced maximum GA in the leaves differing significantly from Biopower. When MN-Slow were used in co-inoculation with TAL-102, 2 folds less GA were produced. Plants inoculated with TAL-102 and CA-18 strains produced 2 folds and 3 folds less GA in leaves than that of Biopower.GA produced by the Grasimi Tika and its pure culture and Biozote and its pure culture were same.

Results demonstrated that plants inoculated with, Biopower showed greater root length than those treated with Biozote and Grasimi Tika. Plants inoculated with pure culture of *Rhizobium* strain MN-Slow either used alone or in co-inoculation with TAL-102 and TAL-102+CA-18 exhibited 1 fold less root length which was significantly lower than that of Biopower (Table V). Inoculation with strain TAL-169 increased root length, significantly greater from that in Biozote. Same results were obtained in case of Grasimi Tika and its pure culture.

Plants inoculated with Biopower showed maximum shoot length, differing significantly from shoot length of plants inoculated with Grasimi Tika and Biozote. Plants inoculated with *Rhizobium* strain MN-Slow exhibited shoot length which was statistically similar with Biopower; however, when used in co-inoculation with CA-18 and TAL-102+CA-18 the shoot length was significantly lower than that of Biopower; however, no significant difference in the shoot length of plants was recorded when inoculated with any of these cultures alone. Co-inoculation of TAL-102 with CA-18 and MN-Slow resulted in higher efficiency. Inoculation with Biozote and TAL-169 as well as Grasimi Tika and SM-101 resulted in statistically same shoot length.

Results revealed that plants inoculated with Biopower showed more than 2 folds greater root dry weight than that of Biozote and about 1 fold greater than that of Grasimi Tika (Table VI). Plants inoculated with pure culture of *Rhizobium* strains used in Biopower showed root dry weight significantly lower than that of Biopower. Plants co-inoculated with TAL-102+MN-Slow, MN-Slow+CA-18, TAL-102 and CA-18 showed statistically similar dry weights. Plants inoculated with TAL-169 and Biozote showed statistically similar results however, *Rhizobium* strain SM-101 treated plants showed root dry weight 1 fold greater than that recorded in the Grasimi Tika.

Table IV. Effects of carrier-based biofertilizers and pure cultures of the strains of *Rhizobium* on the IAA and GA concentration (mg/kg) in mungbean leaves after nodulation (35 DAS)

Treatments	IAA*	GA**
Control (uninoculated)	0.1000 g	1.190 h
Biopower	1.456 a	631.7 b
TAL-102+MN-slow+Ca-18	0.1890 fg	829.6 a
TAL-102+MN-Slow	0.7680 cd	488.1 c
TAL-102+Ca-18	0.6557 de	333.5 ef
MN-Slow + Ca-18	0.1683 fg	384.2 de
MN-Slow	0.9767 fg	779.9 a
TAL-102	0.9613 cd	383.9 de
Ca-18	0.4130 ef	195.2 g
Biozote	0.3720 ef	294.5 f
TAL-169	0.2257 fg	279.4 f
Grasimi tika	0.9970 bc	392.0 de
SM-101	1.299 ab	433.6 cd

LSD value at 0.05=0.3140*, 79.02**; Figures not sharing the same letter differ significantly at p 0.05

Table V. Effects of carrier-based biofertilizers and pure cultures of the strains of *Rhizobium* on the root/shoot length (cm) of mung bean plants

Treatments	Root Length*	Shoot length**
Control (uninoculated)	6.33 g	19.80 f
Biopower	15.73 a	29.83 a
TAL-102+MN-slow+Ca-18	14.97 ab	24.57 c
TAL-102+MN-Slow	14.50 b	26.83 b
TAL-102+Ca-18	11.70 de	21.63 de
MN-Slow + Ca-18	12.00 d	25.00 c
MN-Slow	14.70 b	31.20 a
TAL-102	11.83 de	24.53 c
Ca-18	10.17 f	20.47 ef
Biozote	10.97 ef	22.13 d
TAL-169	12.37 d	22.87 d
Grasimi tika	13.53 c	25.17 c
SM-101	6.33 g	25.47 bc

LSD value at 0.05=0.8944*, 1.453**; Figures not sharing the same letter differ significantly at p 0.05

Shoot dry weight of inoculated plants was significantly higher than that of uninoculated (control) plants. Shoot dry weight was recorded to be maximum in the plants receiving Biopower treatment, which was 1 fold greater than that of Biozote. Plants inoculated with pure culture of *Rhizobium* strain MN-Slow either used alone or in co-inoculation with TAL-102+CA-18, TAL-102 and CA-18 showed dry weight significantly lower than Biopower but, there was significant decline in shoot dry weight when these strains were used alone. Plants receiving TAL-169 treatment showed statistically similar dry weight as recorded in the Biozote but pure culture of strain SM-101 showed dry weight that was significantly higher than that recorded in Grasimi Tika.

Data in Table VII demonstrated that numbers of nodules were recorded to be the maximum in the Biopower treated plants which were statistically similar to that of Grasimi Tika but differed significantly than those recorded in Biozote treatment. Plants inoculated with MN-Slow alone as well as in co-inoculation with TAL-102+CA-18, TAL-102 and CA-18 produced numbers of nodules statistically

Table VI. Effects of carrier-based biofertilizers and pure cultures of the strains of *Rhizobium* on the root / shoot dry weight (g) of mungbean plants.

Treatments	Root Dry wt.*	Shoot Dr wt.**
Control (uninoculated)	0.4000 ef	1.720 f
Biopower	0.9067 a	4.983 a
TAL-102+MN-slow+Ca-18	0.6667 b	4.060 b
TAL-102+MN-Slow	0.4600 def	3.993 bc
TAL-102+Ca-18	0.6200 bc	3.80 d
MN-Slow + Ca-18	0.5067 de	3.923 bc
MN-Slow	0.5233 cd	4.130 b
TAL-102	0.3933 ef	3.430 d
Ca-18	0.3800 f	2.587 e
Biozote	0.3567 f	3.373 d
TAL-169	0.4433 def	3.570 cd
Grasimi tika	0.4733 def	4.230 b
SM-101	0.7233 b	5.053

LSD value at 0.05=0.106*, 0.4409**; Figures not sharing the same letter differ significantly at p 0.05

Table VII. Effects of carrier-based biofertilizers and pure cultures of the strains Of *Rhizobium* on the number of nodules/plant & 100 seed weight of mungbean Plants.

Treatments	Number of nodules*	100SeedWeight**
Control (uninoculated)	5.000 e	2.650 e
Biopower	15.333 a	3.507 a
TAL-102+MN-low+Ca-8	13.333 ab	3.090 bc
TAL-102+MN-Slow	12.000 bc	3.073 bc
TAL-102+Ca-18	11.333 bcd	3.007 bcd
MN-Slow + Ca-18	12.333 abc	3.167 b
MN-Slow	12.000 bc	3.090 bc
TAL-102	11.333 bcd	2.907 cd
Ca-18	8.667 d	2.840 d
Biozote	9.667 cd	3.070 bc
TAL-169	11.33 bcd	3.187 b
Grasimi tika	13.00 ab	3.513 a
SM-101	12.67 abc	3.430 a

LSD value at 0.05=2.831*, 1.006**; Figures not sharing the same letter differ significantly at p 0.05

similar to that of Biopower; however, CA-18 or TAL-102 used alone was much less efficient than used in co-inoculation. Plants inoculated with TAL-169 produced similar numbers of nodules as that of Biozote. Statistically similar results were obtained in, SM-101 and its carrier based biofertilizer, Grasimi Tika treatments.

Weight of 100 seeds increased significantly in the plants inoculated with carrier based as well as pure cultures of *Rhizobium* than that of uninoculated (control) plants. Maximum seed weight was recorded in plants inoculated with Biopower, which was statistically similar with that recorded in Grasimi Tika but differing significantly from that of Biozote. Plants inoculated with *Rhizobium* strain MN-Slow alone or in co-inoculation with TAL-102, CA-18 produced 100 seed weight 1 fold less than that produced by Biopower, however co-inoculation proved better.

Inoculation with *Rhizobium* strain TAL-169 and Biozote produced statistically similar seed weights. Similar results were obtained in pure culture, SM-101 and Grasimi Tika.

Plants inoculated with biofertilizers (Biopower, Biozote, Grasimi Tika) showed statistically similar number of seeds (Table VIII). Plants inoculated with MN-Slow either alone or in co-inoculation with TAL-102 and CA-18 resulted in similar number of seeds as that recorded in

Table VIII. Effects of carrier-based biofertilizers and pure cultures of *Rhizobium* on the number of seeds economic yield (g) of mungbean plants.

Treatments	Number of Seeds**	Economic yield **
Control (uninoculated)	7.667 d	24.60 f
Biopower	11.00 a	38.63 ab
TAL-102+MN-slow+Ca-18	9.667 bc	39.63 a
TAL-102+MN-Slow	9.667 bc	30.73 cdef
TAL-102+Ca-18	10.00 abc	26.07 ef
MN-Slow + Ca-18	9.333bc	30.13 cdef
MN-Slow	10.33 ab	32.67 bcd
TAL-102	9.667 bc	26.40 def
Ca-18	9.000 c	27.63 def
Biozote	10.33 ab	25.83 ef
TAL-169	10.00 abc	31.40 cde
Grasimi tika	10.33 ab	34.13 bc
SM-101	11.00	36.13 bc

LSD value at 0.05=0.1760*, 5.67**; Figures not sharing the same letter differ significantly at p 0.05

Table IX. Effects of carrier- based biofertilizers and pure cultures of strains of Rhizobium on the NO_3-N, and P contents (ppm) of soil after harvesting the mung bean Plants.

Treatments	NO_3-N	P
Control (uninoculated)	3.697 d	0.660 cd
Biopower	10.73 c	2.007 bc
TAL-102+MN-slow+Ca-18	4.613 cd	3.520 a
TAL-102+MN-Slow	7.177 cd	2.380 ab
TAL-102+Ca-18	5.670 cd	1.333 bcd
MN-Slow + Ca-18	10.85 c	1.300 bcd
MN-Slow	9.20 cd	1.801 bc
TAL-102	5.073 cd	1.097 bcd
Ca-18	6.187 cd	1.253 bcd
Biozote	12.04 b	1.110 bcd
TAL-169	8.240 cd	2.500 ab
Grasimi tika	8.450 cd	1.713 cd
SM-101	9.253 cd	2.437 ab

LSD value at 0.05=5.394*, 1.218** ; Figures not sharing the same letter differ significantly at p 0.05

Biopower; however, single inoculation with TAL-102, CA-18 proved less efficient than that of co-inoculation. Inoculation with TAL-169 and Biozote produced numbers of seeds not significantly different from each other. Similarly inoculation with Grasimi Tika and its pure culture SM-101 proved similar pattern.

Maximum yield was recorded in plants inoculated with, Biopower being statistically similar with Grasimi Tika but was 1 fold higher than that recorded in Biozote. Plants inoculated with MN-Slow either alone or in co-inoculation with TAL-102+CA-18 produced yield statistically similar to that of Biopower treated plants. There was no significant difference in the yield produced by Grasimi Tika and its pure culture SM-101 and that of Biozote and its pure culture TAL-169.

Results in Table IX demonstrated that inoculation with either carrier based inocula i.e. biofertilizers or with pure cultures of *Rhizobium* used alone or in co-inoculation exhibited residual higher N and P contents of soil used as culture media as compared to that of un inoculated (control) plants. Among the biofertilizers Biozote resulted in maximum NO_3 -N content of soil; whereas, SM-101 or co-inoculation with TAL-102, MN-Slow and TAL-102, CA-18

and MN-Slow among the pure culture inoculation proved better and maximum NO_3- N content of soil was recorded.

Results also evidenced that inoculation with biofertilizer; Biopower resulted in maximum residual P contents of soil. Among pure cultures of Biopower maximum residual P content was observed in soil treated with MN-Slow+TAL-102+CA-18. Overall P content increased in co-inoculation than alone. Residual P contents were significantly higher in soil receiving pure culture (TAL-169) of biofertilizer Biozote. Similar pattern was observed in Grasimi Tika and its pure culture SM-101.

DISCUSSION

IAA and GA Concentration in Biofertilizers and Pure Cultures of *Rhizobium Leguminosarum.* In the present investigation, biofertilizers (fresh and stored) and their pure cultures were tested for IAA & GA production. Among the biofertilizers maximum IAA and GA concentration was recorded in Biopower. It may be due to the greater efficiency of strains used in Biopower for more hormone production in pure culture as is evident from the results in Table III. This is in accordance with the findings of Rasul *et al.* (1998) and Mehnaz, (2001) who reported that all the strains synthesized more or less IAA and GA in pure culture. It has already been reported that over 80% of the bacteria isolated from rhizosphere can produce IAA, (Arshad & Frankberger, 1998) and GA, Bastian *et al.* (1997). Another possibility for greater hormone produced in the Biopower may be due to the quality of carrier material, which complies with the findings of Graham Weiss *et al.* (1987) who claimed that some carriers are better than others to support the survival and longevity of rhizobia in them.

Minimum IAA & GA reported in Biozote may be due to the less efficient strain TAL-169, having less ability to produce IAA and GA in pure culture than Biopower strains. Also it may be due to poor quality carrier (peat soil) coinciding with the observation of Bajpai *et al.* (1978) who recommended peat soil as carrier for *Rhizobium viceae* only. IAA and GA in Grasimi Tika were more than Biozote but less than Biopower.

IAA and GA contents of leaves before and after nodulation. Inoculation significantly increased IAA and GA contents of leaves before nodulation but there was not significant increase in IAA content but GA content effect showed significant increase than that of control.

Maximum IAA and GA were recorded in leaves of Biopower treated plants, than its pure culture before nodulation in accordance with the findings of Castacura and Venderleyden, (1995). This may be due to stimulatory effect of carrier on microbes, prolonging their life in rhizosphere, increase competitive ability and to face extreme environmental conditions than its pure culture strains (described already in biofertilizers). Co-inoculation increased the IAA and GA contents of leaves then singly applied Rhizobial culture. It might result due to synergistic effects of Rhizobia as reported by Dashti *et al.* (1998).

For measurements made after nodulation IAA content of leaves decreased significantly but GA content increased significantly as compared to that of before nodulation. Among carrier-based biofertilizers, Biopower and Grasimi Tika showed inhibitory effects and Biozote had stimulatory effects on GA production for measurements made after nodulation.

Root/shoot length and dry weights. In the inoculated plants both root/shoot length increased significantly than uninoculated (control). This complies with the findings of Rasul *et al.* (1998) who reported that root-shoot elongation is associated with the production of IAA in early stages and it is evident from Table III & IV that IAA content was increased in inoculated plants as compared to control. Kapulnik *et al.* (1985) also reported that increased root-shoot length after inoculation was due to bacterial phytohormones. Co-inoculation resulted in more root/shoot length than single strain. This may be attributed to synergistic effects (Iruthayathas *et al.,* 1983).

Number of nodules. The number of nodules increased significantly in treatments with pure cultures of *Rhizobium* and carrier based inocula treated plants. It has already been reported by Hunter *et al.* (1994); Number of nodules was reported to be the maximum in Biopower than that of its pure culture treatment. It might have resulted due to more competitive ability of microbes in carrier than in pure culture against native rhizobial population. Since roots are the sites for microbial infection, well-developed root system provides more evidence for infection resulting in greater number of nodules. Furthermore, Biopower and its pure culture appeared to have greater stimulatory effects on root/shoot growth (Table V) as compared to other biofertilizers. Higher concentration of IAA and GA in Biopower, produced by microbes may be another cause for more nodulation as demonstrated by Singh (1993).

Increased number of nodules recorded in co-inoculation may be due to synergistic effects of rhizobia as noted by Yahalom *et al.* (1987), and Iruthayathas *et al.* (1983). Lesser number of nodules produced due to Biozote than that of its pure culture might be due to less IAA and GA production by the microbes and hence reduced growth of root system as compared to that of Biopower.

Yield parameters. Inoculation significantly increased the number of pods, pod length, number of seeds, 100 seed weight and yield than control table VII and VIII. Similar findings were recorded by Provorov *et al.* (1998) and Jain *et al.* (1999).

Yield characters increased significantly in plants treated with Biopower than that of its pure culture. It indicates the rhizobial strains present in the Biopower were more efficient in hormone production (Table II) and biomass production Pal (1986).

Co-inoculation effect of rhizobial strains used in Biopower was noted to be higher than single strain

application. This may be due to synergistic effect as reported by Camacho *et al.* (2001) and Jain *et al.* (1999).

Increase in yield parameters was more favorably affected by the pure culture of strain TAL-169 than its biofertilizer, Biozote. Possibly this may be due to inhibitory effect of carrier material on the multiplication and survival of rhizobia or to their hormone production ability.

NP contents of soil. During the present investigation soil was tested after harvest of plants for residual NP contents. Available N contents increased significantly in inoculated soil than uninoculated control. It may be due to reason that as the microbes fixes nitrogen and this nitrogen fixation suppresses nitrate uptake as demonstrated by Zarin *et al.* (1998) who reported that soil nitrate was higher when hormones were applied to nitrogen fixing chickpea plants. It co relates with maximum nodulation influenced by microbes Singh and Kumar (1989). Moreover, significant increase in N content may be due to the fact that legumes contribute to the total pool of nitrogen in the soil as observed by Ahmad *et al.* (2001). Some nodules get sloughed off from senescing plants at harvest and results in increased NO_3- N and other macronutrients.

Higher P content may be due to inoculation and availability of P nutrients in soil by microbes.

REFERENCES

Agricultural Statistics of Pakistan, 2002-03. *Government of Pakistan, Ministry of Food, Agriculture and Livestock*, p: 19. Food, Agric, Livestock Div, Economic Wing, Islamabad

Ahmad, I. and A. Jan, 2001. Food Legumes. *In: Text Book of Agriculture* pp: 219–24, New Millennium Edition Cropping Technology. National Book Foundation Islamabad

Arshad, M. and Jr.W.T. Frankenberger, 1998. Microbial production of plant growth regulators. *In:* Meeting, B.F. (Ed.), *Soil Microbial Ecology*, pp: 307–47. Mercel Dekker, New York

Bastian, F., A. Cohen, P. Piccoli, V. Luna, R. Baraldi and R. Bottini, 1997. Production of indole–3–acetic acid and gibberellins A_1 and A_3 by *Acetobacter diazotrophicus* and *Herbaspirillum seropidicae* in chemically defined culture media. *Plant Growth Regula.*, 24: 7–11

Camacho. M., C. Santamaria, F. Temprano, D.N. Rodriguez–Navarro and A. Daza, 2001. Co–inoculation with *Bacillus* sp. CECT 450 improves nodulation in *Phaseolus vulgaris. Candian J. Microbiol.*, 47: 1058–62

Costacurta, A. and J. Venderleyden, 1995. Synthesis of phytohormones by plant associated bacteria. *Critical Rev. Microbiol.*, 21: 1–18

Dashti, N., F. Zhang, R. Hynes and D.L. Smith, 1998. Application of plant growth promoting rhizobacteria to *Glycine max* increases protein and dry matter yield under short season conditions. *Pl. Soil*, 188: 33–41

Graham–Weiss, L., M.L. Bennett and A.S. Paau, 1987. Production of bacterial inoculants by direct fermentation on nutrient supplemented vermiculite. *Appl. Environ. Microbiol.*, 53: 2138–40

Hunter, W.J., 1994. Increased nodulation of *Glycine max* by a strain of *Bradyrhizobium japonicum* with altered tryptophan metabolism. *Letters in Appl. Microbiol.*, 18: 340–2

Iruthayathas, E.E., S. Gunasekaran and S. Vlassak, 1983. Effect of combined inoculation of *Azospirillum* and *Rhizobium* on nodulation and N_2– fixation of winged bean and *Glycine max. Scie Hortic (Ams).*, 20: 231–40

Ishizuka, J., 1992. Trends in biological nitrogen fixation research and application. *Pl. Soil*, 141: 197–207

Jain, P.C., P.S. Kushwaha, U.S. Dhakad, H. Khan and S.K. Trivedi, 1999. Response of *Cicer arietinum* to phosphorous and biofertilizer. *Legume Res.*, 22: 241–4

Kapulnik, Y., Y. Okon and Y. Henis, 1985. Changes in root morphology of wheat caused by *Azospirillum* inoculation. *Candian J. Microbiol.*, 31: 881–7

Kettner, J. and K. Droffling, 1995. Biosynthesis and metabolism of Abscisic acid in tomato leaves with Botrytis cineria. *Planta*, 196: 627–34

Lie, J.C., J. Shi, X.L. Zhao, G. Wang, H.F. Yu and Y.J. Ren, 1994. Separation and determination of three kinds of plant hormone by high performance liquid chromatography. *Fenxi–Huaxue.*, 22: 801–4

Lupwayi, N.Z., I. Haque and F.B. Holl, 1997. Strain specific response of *Trifolium semipilosum* to inoculation with *Rhizobium* and significance of water logging in versitols. *Agric. Sci.*, 129: 439–66

Martinez, T., D.L. Rubia, J. Moreno and J. Gonzalez, 1998. Root exudates of *Zea mays* and production of auxins, gibberellins and cytokinins by *Azotobacter chroococcum*. *Pl. Soil*, 26: 881–6

Mehlich, A., 1984. Mehlich 3 soil test extractant. Determination of P, Ca,Mg, K^-. No. 3, North Carolina Soil Test Division, 15: 1409–16

Mehnaz, S., M.S. Mirza, J. Haurat, R. Bally, P. Normand, A. Bano and K.A. Malik, 2001. Isolation and16S rRNA sequence analysis of the beneficial bacteria from the rhizosphere of rice. *Canadian J. Microbiol.*, 47: 110–7

Motsara, M.R., P.B. Bhattacharya and B. Srivastava, 1995. Sources of Nitrogen and the importance of Biofertilizers P. 6. *In*: Biofertilizer Technology, Marketing and usage. Fertilizer development and consultation organization, p. 204–204. A Bhanot Corner, 1–2 Pamposh enclaves, New Delhi, India

Mullen, M.D., D.W. Isreal and A.G Wollum, 1988. Effects of *Bradyrhizobium japonicum* on *Glycine max*. Phosphorus nutrition on nodulation and dinitrogen fixation. *Appl. Environ. Microbiol.*, 54: 2387–92

Pal, P.K., 1986. Impact of Rhizobial strains and micronutrients on grain yield of *Arachis hypogea*., pp: 721–4. *Department Agriculture Chemical Soil. Sci, Environ. Ecol*.

Provorov, N.A., U.B. Saimnazarov, U.I. Bahromov, D.Z. Pulatova, A.P. Kozhemyakov and G.A. Kurbanov, 1998. The effect of *Rhizobium* inoculation on the seed (herbage) production of *Phaseolus aureus* grown at Uzbekistan. *J. Arid. Environ.*, 34: 569–75

Rasul, G., M.S. Mirza, F. Latif and K.A. Malik, 1998. Identification of plant growth hormones produced by bacterial isolates from rice, wheat and kallar grass. *In:* Malik K.A and M.S. Mirza (Eds), *Nitrogen Fixation with non Legumes*, pp. 25–37 Kluwer Publishers, Netherlands

Rengel, Z., 2002**.** Breeding for better symbiosis. *Plant soil.*, 245: 147–62

Samasegaran, P.H., Hoben and J. Halliday, 1982. The NIFTAL (Nitrogen Fixation in Tropical Agricultural Legumes) Manual for methods in Legume–*Rhizobium* Technology. US Agency for International Development, College of Tropical Agriculture and Resources, University of Hawaii.

Serrag, R. and T.R. Sinclair, 1998. Soybean cultivar variability for nodule formation and growth under drought. *Pl. Soil*, 202: 159–66

Singh, T.B., 1993. Effect of growth regulators on nodulation and N_2 fixation in *vigna mungo*. *Comp. Physiol. Ecol.*, 18: 79–82

Singh, T.B. and V. Kumar, 1989. Nodulation and plant growth as influenced by growth regulators in some legumes. *Acta. Bot. Indica.*, 17: 177–81

Smith, A and L. Roberts, 1987. Legume genetic resources for semi arid temperate Environments. *Inter. Cen. Agric. Res. Dry Areas ICARDA, Allepo, Syria.*,: 363

Tien, T.M., M.H. Gaskins and D.H. Hubbell, 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of *pennisetum americanum. Appl. Environ. Microbiol.*, 37: 1016–24

Yahalom, E., Y. Okon and A. Dovrat, 1987. *Azospirillum* effects on susceptibility to *Rhizobium* nodulation and on nitrogen fixation of several forage legumes. *Canadian J. Microbiol.*, 33: 510–4

Zarin, F., A. Bano and M. Aslam, 1998. Effect of plant growth regulators and *Rhizobium* inoculum on N_2 fixation and yield of *Cicer arietinum*. *In*: *Proc*. 7th Int. Symp. of Nitrogen Fixation with non–legumes pp: 103–6. Kluwer Acedemic Publishers, Great Britain

(Received 11 May 2004; Accepted 26 June 2004)