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SYNERGISTIC USE OF *RHIZOBIUM*, COMPOST AND NITROGEN TO IMPROVE GROWTH AND YIELD OF MUNGBEAN (*Vigna radiata*)

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Mungbean is capable of fixing atmospheric nitrogen through *Rhizobium* living in its root nodules. *Rhizobium* inoculation in combination with compost and different levels of mineral nitrogen may improve the performance of mungbean. Treatments were comprised of control (uninoculated), *Rhizobium* combined with compost @ 800 kg ha^{-1} and three levels (50, 75 and 100%) of recommended mineral nitrogen (RMN). Application of compost either in combination with RMN or with *Rhizobium* plus RMN produced significantly higher mungbean yield compared to control. However, the combined application of *Rhizobium*, compost and 75% of the RMN gave maximum increase 18, 82, 87, 89 and 19% in grain nitrogen content, nodule fresh and dry weight, number of nodules and grain yield plant^{-1}respectively, compared to control. Although, the different combinations of RMN along with *Rhizobium* and compost increased net profit but the maximum net profit (Rs. ha^{-1} 20,431) was obtained by utilizing this combination (75% RMN + compost + *Rhizobium*). The significant positive correlation of nodulation with grain yield (r=0.60) and grain N contents (r=0.61) was observed, however nodulation strongly correlated with height (r=0.81). Results demonstrate the potential benefits of using *Rhizobium* along with compost and 75% RMN in order to achieve higher yield of mungbean.
Keywords: Compost, *Rhizobium*, nitrogen, yield, nodulation, mungbean

INTRODUCTION

Mungbean is an important pulse crop that can be grown twice a year, spring and autumn. Its seed is more palatable, nutritive, digestible and non-flatulent than other pulses grown in country. It contains 24.7% protein, 0.6% fat, 0.9% fiber and 3.7% ash (Potter and Hotchkiss, 1997). Besides being a rich source of protein, it maintains soil fertility through biological nitrogen fixation (BNF) (Elahi *et al*., 2004) and thus plays a vital role in increasing yield of crops on sustainable basis (Kannaiyan, 1999). *Rhizobium* inoculation is mandatory when soils have low indigenous or established but inefficient rhizobial population (Araujo *et al*., 1994). In Pakistan, soils have either lacking or very low viable count of effective rhizobia consequently low nitrogen-supplying capacity (Achakzai, 2007). But we are searching for those rhizobial isolates which cause effective nodulation to legume host. Therefore, it is dire need to inoculate legumes with specific *Rhizobium* specie. These bacteria invade the root hairs of mungbean leading to the formation of nodules, where free air nitrogen is fixed. *Rhizobium* inoculation improved nodulation and thus promote the yield of crops (Henzell, 1988). Similarly, Yang *et al*. (2008) reported increase in yield owing to *Rhizobium* inoculation. Composts are known to recover crop production directly and indirectly by improving the soil properties. The use of compost reduces the application of inorganic fertilizers. Its important contribution is the high organic matter fraction and its addition in soil improves soil structure, texture and tilth (Hesse and Misra, 1982; Eneji *et al*., 2001; Singh *et al*., 2001). It has been reported that nodulation, availability and uptake of Zn, Mn and Fe increased by the application of compost (Sahni *et al*., 2008; Shahzad *et al*., 2008; Shukla and Ptyagi, 2009).

Nitrogen (N) is the most essential nutrient element and its adequate supply increases growth and yield of crop. It alters leaf area and net photosynthetic rate because its usage strongly affects plant growth and productivity; therefore, the application of N fertilizer is very important to obtain maximum potential of crops (Novoa and Loomis, 1981). Undoubtedly, higher yield can be obtained by the application of chemical fertilizers as they are instant source of nutrients for crops, but during the last couple of years, the stagnant yields have been obtained and no more increase in crop yields were observed even when fertilizers were applied at optimum level. Moreover, high cost energy inputs required for manufacturing of chemical fertilizers and their shortage in markets diverts the attention of farmers to other cheap fertilizer sources (i.e. organic and bio-fertilizers). The integrated use of organic, inorganic and bio-fertilizers may be an alternative approach to increase stagnant yield of legumes in arid to semiarid climate of Pakistan. It has been reported that combined application of N and *Bradyrhizobium* increased yield of mungbean (Mozumder *et al*., 2003). Moreover, the combined use of N fertilizers at the rate of 50 kg ha^{-1} and *Rhizobium* inoculation produced highest grain yield of legumes (Shah *et al*., 2000; Ashraf *et al*., 2003; Javid *et al*., 2006). The significant increase in grain yield of beans in response to the application of manures and N fertilizer was reported (Otieno *et al*. 2007). Keeping in view the above discussion, it is obligatory to search out innovative alternatives to improve crop productivity on sustainable basis. The objectives of present study were to 1) determine the response of *Rhizobium* under variable nitrogen levels and 2) evaluate efficiency of compost alone and in combination with *Rhizobium* and different levels of recommended mineral nitrogen (RMN) to improve mungbean productivity.

MATERIALS AND METHODS

***Collection of seed, strain and compost*:** Mungbean variety NM-98 was collected from the Pulses Division of Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad. The pre-isolated strain of *Rhizobium* was collected from Soil Microbiology and Biochemistry Laboratory, Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad. Freshly prepared fruit leaves compost was collected from the composter unit where it is prepared on local scale. Briefly, the agricultural waste (crop leftover, fruit and vegetable waste) was collected and piled at the research farm of Institute of Soil & Environmental Sciences, where it was processed mechanically (decomposed, dried and finally grinded) to convert into compost containing 1.96% N, 1.54% P and 1.98% K.

***Pot trial*:** Pot experiment was conducted in the wire house of Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, to evaluate the response of mungbean to *Rhizobium* inoculation along with compost and RMN (as urea @ 25 kg ha^{-1}). Each pot has (20×30 cm dimension), its inner wall aligned with polythene sheath to avoid loss of water and filled with 12 kg soil. Recommended quantities of phosphorus and potassium as single super phosphate and potassium sulfate, respectively, was applied @ 60 and 50 kg ha^{-1}, respectively at the time of sowing. *Rhizobium* combined with compost at the rate of 800 kg ha^{-1} and three levels (50, 75 and 100% of recommended dose) of mineral N was applied. A control without inoculation and compost was included for comparison. The experiment was laid out in completely randomized design (CRD) with six replications.

The inoculum was prepared by using yeast extract manitol (YEM) broth media in conical flask. It was incubated in orbital shaking incubator at 28 ± 1 ºC for three days. The inoculum containing 10^7 - 10^8 CFU mL^{-1} was achieved by dilution with sterilized deionized water on the basis of optical density (OD); for uniformity of rhizobial culture, OD of rhizobial culture was determined using spectrophotometer at 550 nm to maintain cell density of culture at 10^7 - 10^8 CFU mL^{-1}(OD = 0.5, Anjum, 2011). Mungbean seeds were treated with broth culture of rhizobial strain, sterilized peat and 15% sugar solution following 4:5:1ratio. Autoclaved broth (without bacterial culture) peat and sugar mixture was used to treat seeds for control treatment. Seeds were sown according to treatments by using six seeds per pot which were thinned to three plants after germination. All agronomic practices were kept uniform during all growth stages of mungbean. Data regarding nodulation was recorded at flowering stage using three replicates while yield and other growth parameters recorded at maturity from left over replicates. Table 3 shows the economic analysis; cost of production was estimated according to Anjum (2011).

***Grain N content*:** Plant samples were oven dried in oven at 70 ºC for 48 hours and grinded in rotary mil (model Polymix PX-MFC, Switzerland). The ground material (0.1 g) was digested with sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) according to the method of Wolf (1982). Grain nitrogen was determined by Kjeldahl method.

***Statistical analysis*:** Analysis of variance was performed according to Steel *et al*. (1997). Treatments means were compared by least significance difference test (LSD) at $p<0.05$ level of significance using STATISTIX, v8.1. Pearson correlation between different parameters of mungbean was determined using the same software at $p<0.01$ and $p<0.05$.

RESULTS

***Nodulation*:** Lowest number of nodules was recorded in non-inoculated plants which received optimal nutrition of NPK. Addition of compost at the rate of 800 kg ha^{-1} increased nodulation, nodule fresh and dry weight by 40, 42 and 42%, respectively, as compared to reference treatment (Table 1). The inoculation of *Rhizobium* in combination with compost and 50, 75 and 100% of RMN enhanced 73, 89 and 78% nodulation, respectively, compared to reference treatment (control). The treatment combination (compost, *Rhizobium* and 75% of RMN) produced significantly ($p<0.05$) higher nodulation and nodule fresh as compared to control and sole application of compost. Maximum nodule dry weight achieved by the application of 75% of RMN, compost and *Rhizobium* inoculation but it was non-significant ($p<0.05$) with treatment received 50% of RMN,compost and *Rhizobium* inoculation.

***Plant growth*:** Plant height increased by the integrated use of *Rhizobium*, compost and mineral nitrogen (Table 1). The sole application of compost enhanced plant height by 51% compared to reference control and showed highly significant results ($p<0.05$). The maximum significant ($p<0.05$) increase in plant height was observed by the combined application of compost, *Rhizobium* and 100% of RMN compared to other treatments. However, the addition of reduced N rates (50 & 75%) along with *Rhizobium* and compost also showed significant ($p<0.05$) increased plant height by 61 and 76%, respectively, compared to reference control.

Table 1. Effect of Rhizobium inoculation, compost and mineral nitrogen on plant height, number of nodules, nodules fresh and dry weight, number of pods, yield and grain nitrogen contents of mungbean (values having different letter (s) within a column differ significantly p<0.05)

Treatments	Number of nodules plant^{-1}	Nodules fresh weight (mg)	Nodules dry weight (mg)	Plant height (cm)	Number of pods plant^{-1}	Grain yield (g plant^{-1})	Biological yield (g plant^{-1})	N in grains (%)
T_1	15.00 d	13.90 d	1.43 c	11.90 d	9.00 d	3.71 e	16.83 d	1.87 c
T_2	21.00 c	19.77 c	2.03 b	18.00 c	12.00 c	4.30 b	20.68 c	2.17 a
T_3	26.67 ab	20.23 bc	2.10 b	22.70 a	15.00 a	4.15 c	26.86 a	2.18 a
T_4	26.00 b	22.07 b	2.36 ab	19.20 c	13.00 bc	4.03 d	24.07 b	2.04 b
T_5	28.33 a	25.33 a	2.67 a	21.00 b	14.00 ab	4.43 a	24.91 ab	2.20 a
LSD (0.05)	1.94	1.88	0.35	1.25	1.82	0.11	2.15	0.09

Treatments description: control 100% RMN @ 25 kg ha^{-1} (T_1), compost @ 800 kg ha^{-1} + 100% RMN (T_2), compost + *Rhizobium* + 100% RMN (T_3), compost + *Rhizobium* + 50% RMN (T_4), compost + *Rhizobium* + 75% RMN (T_5). All the treatments received recommended doses of PK @ 60 and 50 kg ha^{-1}, respectively.

Plant yield**:** Mungbean yield (number of pods, grain and biological yield i.e. fruit + stem) increased by the addition of compost, mineral N and *Rhizobium* inoculation (Table 1). Lowest number of pods, grain and biological yield was recorded in reference control while it was being nourished by recommended mineral NPK fertilizers. Furthermore, the addition of compost to the basal dose of NPK increased 33, 16 and 23% number of pods, grain and biological yield, respectively, in comparison to reference control. The treatment combination of compost, *Rhizobium* and 100% of RMN produced significantly ($p<0.05$) higher number of pods and biological yield as compared to all other treatments but it was statistically at par with the treatment combination of compost, *Rhizobium* and 75% of RMN. Grain yield was significantly ($p<0.05$) enhanced by the combined application of compost, *Rhizobium* and 75% of RMN which was 3 and 19% higher than recommended NPK plus compost and recommended NPK (control), respectively. There was 14% higher grain yield recorded in treatment received 100% N plus compost which was significant ($p<0.05$) from all other treatments except treatment received combined application of compost, *Rhizobium* and 75% of RMN.

Grain N contents**:** Grain N content was significantly ($p<0.05$) increased with the integrated use of *Rhizobium*, compost and mineral N (Table 1). The grain N contents recorded were 2.17% in the treatment received compost at the rate of 800 kg ha^{-1} in combination with RMN which was significantly higher than the grain N contents (1.87%) recorded in control treatment. *Rhizobium* inoculation along with different N levels and compost further increased grain N contents significantly ($p<0.05$) compared to un-inoculated control. It seems from the results that different treatments had differential effects on grain N contents but the maximum N contents (2.20%) were observed in the treatment which received *Rhizobium* + compost + 75% RMN and it was statistically non-significant ($p<0.05$) with the treatments received compost + RMN (2.17%), and *Rhizobium* + compost + 100% RMN (2.18%).

Correlation parameters**:** Positive correlation was found between plant height, number of pods and nodules, grain yield and N content (Table 2). Highly significant ($p<0.01$) relation was observed in plant height and number of pods while pods was non-significantly ($p<0.05$) related with grain yield. Nodulation was significantly ($p<0.01$) related with plant height, number of pods, grain yield and N contents indicates importance of *Rhizobium* inoculation that fixed atmospheric N and improved these parameters.

Table 2. Correlation between different parameters of mungbean, n=15

	No. of Pods	No. of nodules	Grain yield	Grain No.
Plant height	0.85*	0.81*	0.55**	0.66*
No. of pods		0.75*	0.46^{ns}	0.54**
No. of nodules			0.60**	0.61**
Grain yield				0.82*

*$p<0.01$, **$p<0.05$, ^{ns}non-significant

Economic analysis**:** The variable response of treatments was observed during their economic analysis (Table 3). Results revealed that the treatment received *Rhizobium* inoculation in combination with compost and 75% RMN gave maximum net profit (Rs. ha^{-1} : 20,431) than all other treatment combinations. It followed by the treatment received compost in combination with recommended NPK. Results showed no difference in total expenses of treatments received compost alone or in combination with *Rhizobium* inoculation and different N levels (50, 75 and 100% RMN) whereas greater variations were observed in total income and net profit compared to reference control.

Table 3. Economic analysis (Rs. ha^{-1}) of mungbean under different treatment combinations

Parameters	T_1	T_2	T_3	T_4	T_5
Seed bed preparation	4000	4000	4000	4000	4000
Seed	1500	1500	1500	1500	1500
Irrigation	1100	1100	1100	1100	1100
Pesticide	1000	1000	1000	1000	1000
N as Urea	1136	1136	1136	568	852
P as SSP	5647	5647	5647	5647	5647
K as SOP	5600	5600	5600	5600	5600
Inoculum	-	-	150	150	150
Compost	-	4000	4000	4000	4000
Total cost	19983	23983	24133	23565	23849
Grain yield (kg ha^{-1})	618	717	692	672	738
Total income	37080	43020	41520	40320	44280
Net Profit	17097	19037	17387	16755	20431

Fertilizer cost was calculated in this study according to the rates listed by National Fertilizer Development Center (Rs. bag^{-1}: Urea 1045; SSP 848; SOP 2800) during the year 2010-11. The cost of compost was calculated according to native rates (Rs. 250/50 kg). The market rate of mungbean grain was (Rs. kg^{-1}60). Treatments description: control 100% RMN @ 25 kg ha^{-1} (T_1), compost @ 800 kg ha^{-1} + 100% RMN (T_2), compost + *Rhizobium* + 100% RMN (T_3), compost + *Rhizobium* + 50% RMN (T_4), compost + *Rhizobium* + 75% RMN (T_5). All the treatments received recommended doses of PK @ 60 and 50 kg ha^{-1}, respectively.

DISCUSSION

Our results inferred positive role of *Rhizobium* inoculationon nodulation of mungbean. It might be due to positive symbiotic association of legumes with *Rhizobium*. This kind of positive legume *Rhizobium* association is well documented and recent reports confirmed its positive role on yield and nodulation of different legumes (Togay *et al*., 2008; Ahmad *et al*., 2013). This association brought number of changes in legumes but nodulation is most important which has central position in their developmental growth stages through N_2- fixation from the atmosphere. Each legume released specific exudates which attract specific organisms from rhizosphere towards roots. The present study investigated the effect of different combinations of N along with compost and *Rhizobium* on nodulation, growth and yield of mungbean. Seed inoculation with *Rhizobium* significantly increased plant growth, yield and nodulation of mungbean. Similar results were reported earlier that plants inoculated with *Rhizobium* spp. increased number of nodules (Togay *et al*., 2008). Lowest nodulation in non-inoculated control elucidates decreased activity of native rhizobia but addition of compost and *Rhizobium* increased nodulation. The increased *Rhizobium* activity in root zoon might be due to addition of compost which may act as a carbon and nutrient source for its proliferation there by nodulation was increased. According to Otieno *et al*. (2007) nodulation increased due to slow mineralization of N from compost. On the other hand, nodulation inhibited when higher rate of N was applied as compared to lower rates. It may cause inactivity of inoculated *Rhizobium* to fix biological N in the presence of high amount of N. Actually the NO_3-N does not allow the legumes to fix atmospheric N, because plant needs greater energy to fix atmospheric N than to utilize NO_3 (Zahran, 1999). Moreover, the presence of excess NO_3 level affects *Rhizobium* activity in soil by inhibiting lectin (compound that attract *Rhizobium* towards legume, Dazzo and Hubbell, 1975). Our results are in line with the results of (Mozumder *et al*., 2003; Otieno *et al*., 2007) who reported decreased nodulation due to higher rate of N application. The exogenous application of N may raise the soil N to a level that may reduce nodulation (Laws and Graves, 2005). Nodules number, fresh and dry weight increase might be due to compost which served as source of C, N, P and micronutrients for the native and exogenously inoculated rhizobia as well as for mungbean. Nitrogen applied in combination with *Rhizobium* increased nodulation in legumes (Rashid *et al*., 1999), nodules weight (Mozumder *et al*., 2003), greater nodule weight at lower than higher N fertilization in combination with *Bradyrhizobium* (Mozumder *et al*., 2003). Plant height increased by the inoculation of *Rhizobium* (Togay *et al*., 2008; Ahmad *et al*., 2013) and *Rhizobium* plus N fertilization (Khalil *et al*., 1989; Rahman *et al*., 2002).

The combined addition of mineral N, compost and *Rhizobium* increased yield indicated positive role of these treatments on mungbean. Number of pods increased by N fertilization (Pateland Parner, 1991; Anjum *et al*., 2006); combined use of N and inoculation (Basu and Bandyopadhyay, 1990; Rashid *et al*., 1999; Rahman *et al*., 2002) and sole *Rhizobium* inoculation (Ali *et al*., 2000; Ahmed *et al*., 2007; Ahmad *et al*., 2013). Our results are in line with Rahman *et al*. (2002) who reported that number of pods, yield and N contents increased by the combined application of chemical fertilizers and *Rhizobium* inoculation. Moreover, Chatterjee and Bhattacharjee (2002) reported that the grain yield was significantly higher over control in plants inoculated with *Rhizobium* strain. Similarly, Kumarand

Chandra (2008) reported that the inoculation enhanced the grain N and James *et al*. (2007) found higher grain N by applying nitrogenous fertilizer. Compost improved all the parameters under study it might be due to improved nutrition (Buchgraber, 2000); water holding capacity, increased aggregate stability (Van-Camp *et al*., 2004); reduction of pH which solubilizes the bound nutrients into plant available form and increases biological activity of soil (Fließbach *et al*., 2000; Van-Camp *et al*., 2004); increases yield of crops (Reider *et al*., 2000). In arid conditions it is important to incorporate compost to increase organic matter and moisture contents of the soil (Nandwa, 1995) leading to increase *Rhizobium* activity (Otieno *et al*., 2007). Our results showed that the addition of compost increased nodulation, plant growth and yield. It was reported that compost contained different nutrients especially N and P which may cause increase in nodulation and grain yield (Otieno *et al*. 2007). Moreover, economic analysis shows the importance of compost incorporation in improving grain yield of mungbean leading to better output in terms of net profit (Table 3). Further, inoculation of *Rhizobium* along with compost and different rates of RMN increased the net profit of the farmer. Similar results were obtained by Anjum (2011) by *Rhizobium* inoculation along with recommended or half dose of nitrogen. The economic analysis revealed that by utilizing this treatment combination (*Rhizobium*, compost and 75% RMN) maximum profit was obtained and save 25%

Conclusion: The combined application of compost, *Rhizobium* and 75% of RMN contributed higher output of mungbean. However, addition of compost in combination with recommended NPK could also be useful to improve grain yield and N content which might be due to more availability of nutrients and water under compost application. Farmers can save 25% N by applying aforementioned combination to get higher yield on sustainable basis and can catch maximum profit. Field trial should be conducted to further explore the suitability and rational application of proposed combination.

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