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INFLUENCE OF GROWTH STIMULANTS WITH *RHIZOBIUM* INOCULATION ON THE YIELD OF MUNG BEAN (*Vigna radiata* L.)

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Rhizobium has unique ability to form nodules in legumes, served marvellously in the agricultural systems. Plant hormones like auxins, gibberellins, cytokinins etc. influenced the growth of plants or parts of plants. Although plants synthesize auxins endogenously yet exogenous application has more assenting effects on plants. Exogenous application of auxins can affect the endogenous level under sub-optimal conditions. Studies were planned to assess impact of exogenously applied Indole acetic acid (IAA) and L-tryptophan (L-TRP) with and without *Rhizobium* inoculation on the yield of mung bean at Soil Bacteriology Section, Faisalabad. Fertilizer dose i.e. 30-60 kg NP ha^{-1} was applied while IAA and L-TRP @ 10^{-5} M following two application methods i.e. seed soaking and foliar spray followed by completely randomized design (CRD). *Rhizobium* was isolated from mung bean and screened out for auxin biosynthesis potential and for other biochemical tests. Results (mean of two years) revealed that *Rhizobium* inoculation significantly improved the yield components of mung bean with and without exogenous application of IAA and L-TRP. IAA and L-TRP applied either by seed soaking or foliar spray with *Rhizobium* inoculation proved better than their respective controls. *Rhizobium* inoculation produced significantly higher pod and straw yield i.e. 29.65, 43.37 with L-TRP and 28.95, 43.55 g pot^{-1} with IAA, respectively. *Rhizobium* inoculation produced higher nodules 69, nodule mass 0.458 g pot^{-1} with L-TRP and 63 and 0.462 g pot^{-1} with IAA, respectively. Higher grain and plant N was observed with seed soaked L-TRP with *Rhizobium* i.e. 3.72 and 1.203 % while with IAA i.e. 3.70 and 1.183%. Similarly, higher grain and plant P was observed with seed soaked L-TRP with *Rhizobium* inoculation. *Rhizobium*inoculation improved the soil N and available P with L-TRP and IAA. Auxin (IAA) and its precursor L-TRP affected the mung bean growth parameters and effect was more pronounced with *Rhizobium* inoculation and seed soaking proved better option than foliar spray.
Keywords: IAA, L-TRP, *Rhizobium*, seed soaking, foliar spray, mung bean.

INTRODUCTION

Pulses are marvelous source of proteins and play vital role in the nutritious requirements of burgeoning population. Mungbean is grown in spring and summer season and have less water requirements compared to other summer crops. Mungbean is drought resistant crop and can survive under adverse conditions and in rain fed tracts (Anjum *et al.*, 2006).

Indole-3-acetic acid (IAA) is the most profusely produced auxin influenced the plant growth. Plants produced IAA due to the transamination and decarboxylation reactions of L-TRP and IAA has profound influence on plants such as phototropism, geotropism, fruit set and abscission, enhanced cell division of meristematic cells (Sachdev *et al.*, 2009). Nodule formation is typically a characteristic of *Rhizobium*, the microbe responsible for nodule formation in legumes. Studies revealed that nodule formation also influenced by the auxins (Tao *et al.*, 2008). It was observed that hormones played indispensable role to initiate the nodulation process and enhanced nitrogenase activity in nodules. The biosynthesis of auxins is directly correlated with the plant growth promotion (Idris *et al.*, 2007; Tsavkelova *et al.*, 2007).

L-tryptophan (L-TRP) is an efficient precursor and responsible for biosynthesis of auxins (Khalid *et al.*, 2001; Khalid *et al.*, 2004). Application of L-TRP improved the growth and yield of plants through biosynthesis of auxins and its addition to bacterial cultures enhanced the IAA level (Khalid *et al.*, 2001). Exogenous application of IAA can change the endogenous levels and thus alter the plant ontogeny (Frankenberger and Arshad, 1995; Khalid *et al.*, 2001).

The development of symbiosis between species of *Rhizobium* and legumes has played a marvelous role for sustainable agriculture. *Rhizobium* not only involves in nitrogen fixation but also take part in production of hormones and phosphate solubilization (Zahran, 2001). Plants produced auxins indigenously. Both processes of LCO's (lipo-chitin oligosaccharide) and auxin biosynthesis are triggered by flavonoids, the chemical signals produced by plants (Mehboob *et al.*, 2008). Kamilova *et al.* (2006)

demonstrated that microbes used L-TRP for auxin biosynthesis from the plant root exudates resulted in improved plant growth. Many workers reported that production of IAA was enhanced by the application of L-TRP (Khalid *et al.,* 2001; Asghar *et al.,* 2002; Zahir *et al.,* 2010). The growth medium supplemented with L-TRP produced more IAA (Datta and Basu, 2000; Patten and Glick, 2002).

Microbes belong to the genus *Rhizobium* are very well studied due to its marvelous role of N_2-fixation in legumes and in non-legumes also showed promise for its phosphate solubilization, production of growth hormones and biocontrol feature (Inam-ul-Haq *et al*., 2009; Zahir *et al.,* 2010; Qureshi *et al.,* 2012; Mia *et al.,* 2012).

Studies demonstrated that that exogenous application of growth hormones improved the growth and crop yields (Frankenberger and Arshad, 1995; Asghar *et al.,* 2002; Zahir *et al.,* 2004; Zahir *et al.,* 2010). Different methods of application of growth hormones were reported in literature like seed soaking, foliar spray and soil application (Hye *et al.,* 2002; Fatima *et al.,* 2008; Bano *et al.,* 2009). Plant hormones affect the plant growth and development either positively (growth promoter) or negatively (growth retardant). The effect of plant hormones is largely depended on concentration, method of application and time of application (Arshad and Frankenberger, 1991). The method of application of plant hormones depends upon the type of hormone and its time of application must be kept in consideration. Foliar spray improve the plant height as compared to seed soaking while seed soaking improve the germination and seedling vigor while soil application with water have least loss and easily accessible to vascular tissue of roots but on the other hand cost effective in nature (Unamba *et al.,* 2009).

Present studies were conducted to assess the influence of auxin (IAA), its precursor (L-TRP) with and without *Rhizobium* inoculation and two methods of application i.e. seed soaking and foliar spray was compared.

MATERIALS AND METHODS

***Isolation of Rhizobium sp*:** Plants of mung bean were collected from Soil Bacteriology Section, AARI Faisalabad and detached nodules were surface-sterilized (Russell *et al.,* 1982). Nodules were crushed in aseptic conditions and obtained suspension was streaked out on yeast extract mannitol agar medium (YMA) (Vincent, 1970). Rhizobial growth was transferred again and again on YMA medium to purify the growth. Different dyes were used to identify the behavior of isolates i.e. Congo red, Bromothymol blue (BTB) and stained for their gram reaction (Keneni *et al.,* 2010). The rhizobial growth was re-streaked persistently to obtain pure cultures. Thus, three isolates were purified from the nodules of mungbean and selected on the basis of IAA content and stored at 4±1°C on slants.

***Biochemical tests*:** The purified isolates were screened for Gram reaction, catalase test (Addition of 3% H_2O_2 to bacterial growth and if air bubbles comes out results in positive test), urease (Urea broth added with phenol red turns pink show positive urease), starch hydrolysis (Gram iodine forms complex with starch of blue to brown colour and if microbe clear area that means positive starch hydrolysis) and citrate utilization (Sodium citrate is used as sole carbon source and NH_4^+ as nitrogen source and bromothymol blue as indicator, medium turns from green to blue results in positive test) (Krieg and Holt, 1984). Auxin biosynthesis potential of isolates i.e.Mb_1, Mb_2 and Mb_3 was determined with and without L-TRP using Salkowski's reagent (98 mL of 35% $HClO_4$ + 2 mL of 0.5M $FeCl_3$) as indole-3-acetic acid (IAA) equivalents (Sarwar *et al.,* 1992). Isolates having the highest IAA production potential was selected for pot studies.

***Inoculum preparation and pot experiments*:** After preliminary screening of isolates, (Mb_3) was selected, multiplied in broth of yeast extract mannitol (YMB) and incubated at 28±2°C for 3-5 days to get the optimum growth i.e. optical density of 0.5. Inoculum was prepared by mixing 20 mL broth and 200 g of sterilized peat collected from 'Changa Manga' forest (Qureshi *et al.,* 2012; Naveed *et al.,* 2014).

Pot studies were conducted in two different years on medium textured soil [pH 7.95, EC 1.24 dS m^{-1}, N 0.037%, available P 9.33 mg kg^{-1} and organic matter 0.76%]at Soil Bacteriology Section, AARI, Faisalabad. Recommended fertilizer i.e. 30-60 kg NP ha^{-1} was applied basal as urea and single super phosphate (SSP) following completely randomized design (CRD) with four replications. The surface sterilization of mung bean seeds cv. AZRI-2006 was carried out as described by Russell *et al*. (1982). Seeds of mung bean were soaked in distilled water (treated as control), aqueous solution of IAA & L-TRP (each at 10^{-5} M) for three hours (Qureshi *et al.,* 2012; Zahir *et al.,* 2010; Naveed *et al.,* 2014). These growth regulators were also used as foliar spray after three weeks of germination as per treatment (each at 10^{-5} M concentration). On the basis of auxin biosynthesis potential and biochemical tests, Mb_3 was used for experimentation.

At flowering stage, plants of two repeats were up-rooted for determination of number of nodule and nodular mass, root length/mass. Data regarding pod, straw yield, NP-content in grains and straw was determined from the rest of two repeats. Postharvest soil N was determined by Kjeldhal method (Bremner and Mulvany, 1982) and available P by modified Olsen method (Olsen and Sommers, 1982). The statistical analysis of average of two years data was carried out following CRD (Steel *et al.,* 1997). The differences among the means were compared by the Duncan's multiple range tests (DMR) (Duncan, 1955).

RESULTS

Results clearly showed that *Rhizobium* inoculation and exogenous application of IAA and L-TRP significantly enhanced the yield attributes of mung bean. It was also noticed that exogenous application of IAA and L-TRP enhanced the crop yields and more improvement was observed with *Rhizobium* sp inoculation.

The results showed that IAA and its precursor L-TRP with *Rhizobium* sp inoculation influenced the pod and straw yield (Table 2). *Rhizobium* sp inoculation clearly improved the pod and straw yield as compared to control. Seed soaking of IAA and L-TRP affected the pod and straw yield positively as compared to foliar spray. *Rhizobium* inoculation produced pod and straw yield (29.65; 43.37 g pot^{-1}) with L-TRP followed by (28.95; 43.55 g pot^{-1}) with IAA applied as seed soaking. Percent increase in pod and straw yield with

Table 1. Some important characteristics of isolates used in the study.

Isolates	IAA equivalents (µg mL^{-1}) L-TRP [-]	IAA equivalents (µg mL^{-1}) L-TRP [+]	BTB test	Congo red test	Urease Test	Starch hydrolysis	Citrate utilization
Mb_1	4.91	7.52	+ve	+ve	-ve	-ve	-ve
Mb_2	5.28	7.90	+ve	+ve	-ve	+ve	-ve
Mb_3	5.75	8.36	+ve	+ve	+ve	+ve	+ve

LSD = 1.15

Table 2. Effect of IAA and L-TRP with and without inoculation on mungbean.

Treatments	Pod Yield (g pot^{-1})		Straw Yield (g pot^{-1})	
	Un-inoculated	Inoculated	Un-inoculated	Inoculated
Control	21.55 d*	23.38 c	22.72 e*	25.25 e
IAA (SS*)	23.57 c	28.95 a	34.63 bc	43.55 a
IAA (FS*)	25.20 bc	26.22 b	30.28 d	34.93 bc
L-TRP (SS)	26.43 b	29.65 a	36.52 b	43.37 a
L-TRP (FS)	24.68 bc	25.82 b	33.13 cd	37.58
LSD	1.83		2.95	

SS*: Seed soaking; FS*: Foliar spray, *Means sharing the same letter(s) in a column do not differ significantly at $p<0.05$ according to Duncan's Multiple Range Test.

Table 3. Effect of IAA and L-TRP with and without inoculation on mungbean plant matter NP content.

Treatments	Plant N-content (%)		Plant P-content (%)	
	Un-inoculated	Inoculated	Un-inoculated	Inoculated
Control	1.070 c*	1.090 c	0.195 f*	0.227 e
IAA (SS*)	1.141 b	1.183 a	0.245 bcd	0.265 a
IAA (FS*)	1.135 b	1.151 b	0.232 de	0.249 bc
L-TRP (SS)	1.187 a	1.203 a	0.257 ab	0.270 a
L-TRP (FS)	1.138 b	1.153 b	0.240 cde	0.249 bc
LSD	0.026		0.013	

SS*: Seed soaking; FS*: Foliar spray, *Means sharing the same letter(s) in a column do not differ significantly at $p<0.05$ according to Duncan's Multiple Range Test.

Table 4. Effect of IAA and L-TRP with and without inoculation on mungbean grain NP content.

Treatments	Grain N-content (%)		Grain P-content (%)	
	Un-inoculated	Inoculated	Un-inoculated	Inoculated
Control	3.49	3.52	0.295 g*	0.315 f
IAA (SS*)	3.66	3.70	0.370 bcd	0.388 ab
IAA (FS*)	3.59	3.65	0.360 de	0.371 bcd
L-TRP (SS)	3.69	3.72	0.379 abc	0.392 a
L-TRP (FS)	3.58	3.62	0.351 e	0.364 cde
LSD	NS		0.018	

SS*: Seed soaking; FS*: Foliar spray, *Means sharing the same letter(s) in a column do not differ significantly at $p<0.05$ according to Duncan's Multiple Range Test.

Table 5. Effect of IAA and L-TRP with and without inoculation on soil N and available P of mung bean.

Treatments	Soil N (%)		Available P (mg kg^{-1})	
	Un-inoculated	**Inoculated**	**Un-inoculated**	**Inoculated**
Control	0.040 f*	0.041 ef	9.82 e*	10.55 cde
IAA (SS*)	0.047 bc	0.051 a	12.05 ab	13.46 a
IAA (FS*)	0.043 de	0.046 bc	10.34 de	11.46 bcd
L-TRP (SS)	0.048 b	0.051 a	12.64 ab	13.97 a
L-TRP (FS)	0.045 cd	0.047 b	10.79 cde	11.54 bcd
LSD	0.002		1.41	

SS*: Seed soaking; FS*: Foliar spray, *Means sharing the same letter(s) in a column do not differ significantly at p<0.05 according to Duncan's Multiple Range Test.

Rhizobium inoculation along with IAA as seed soaking (22.83, 25.75%) and with L-TRP (12.18, 18.75%) while IAA applied as foliar spray with inoculation (4.05, 15.36%), and with L-TRP (4.62, 13.43%) than respective un-inoculated ones, respectively.

Data regarding plant and grain NP-content (Table 3, 4) revealed that IAA and its precursor L-TRP with *Rhizobium* inoculation affected mung plant nutrient content positively. Exogenous application of IAA and L-TRP with *Rhizobium* inoculation improved the plant and grain NP content either applied as seed soaking or foliar spray. Seed soaking of IAA and L-TRP improved the plant NP content as compared to foliar spray. L-TRP and IAA applied as seed soaking showed maximum plant NP and grain NP content with *Rhizobium* inoculation i.e. 1.203, 1.183% and 0.270, 0.265% and 3.72, 3.70% and 0.392, 0.388% as compared to un-inoculated control 1.070 and 0.195% and 3.49 and 0.295%, respectively. Auxin as IAA and its precursor as L-TRP applied as seed soaking with *Rhizobium* inoculation increased plant N and P content (8.53 and 10.37%; 16.74 and 18.94%) and grain N and P content (5.11 and 5.68%; 23.17 and 24.44%) than *Rhizobium* inoculation alone.

Data related to post harvest soil N and available P (Table 5) revealed that *Rhizobium* inoculation enhanced the soil N and available P and the effect was more pronounced with exogenous application of IAA and L-TRP. Results depicted that IAA and L-TRP applied as seed soaking with inoculation improved the soil N which was at par i.e. 0.051%. Whereas L-TRP and IAA applied as seed soaking with inoculation enhanced the available P content i.e. 13.97 and 13.46 mg kg^{-1} soil compared to un-inoculated control i.e. 9.82 mg kg^{-1} soil. Although foliar spray with and without inoculation affected the soil N and available P positively yet the effect was more pronounced with seed soaking. Percent increase in soil N and available P with IAA (8.51 and 11.70%) and with L-TRP (6.25 and 10.52%) as seed soaking along with inoculation than respective un-inoculated ones, respectively.

Data related to root parameters like number of nodules pot^{-1}, nodule mass pot^{-1}, root length and mass with exogenous application of IAA and L-TRP are presented in (Fig. 1, 2, 3, 4).

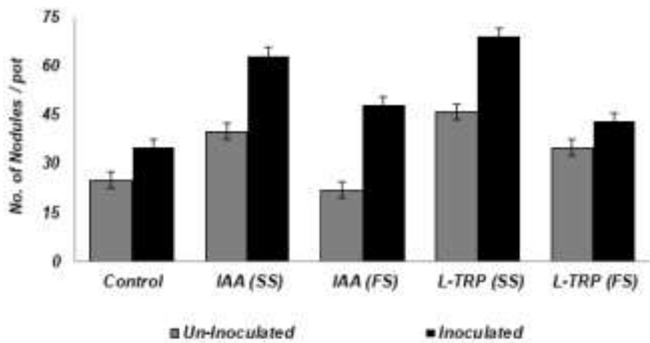

Figure 1. Effect and IAA and L-TRP on the number of nodules with and without inoculation on mung bean.

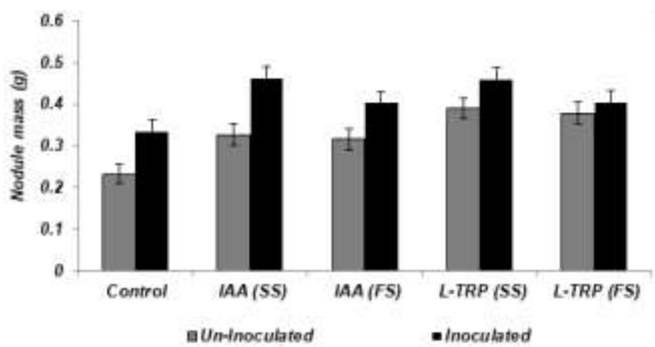

Figure 2. Effect and IAA and L-TRP on the nodular mass with and without inoculation on mung bean.

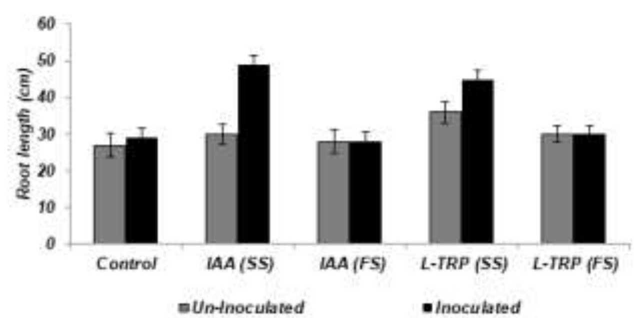

Figure 3. Effect and IAA and L-TRP on the root length with and without inoculation on mung bean.

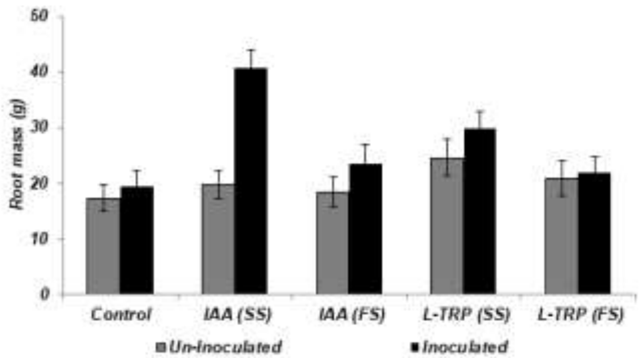

Figure 4. Effect and IAA and L-TRP on the root mass with and without inoculation on mung bean.

Application of IAA or L-TRP as seed soaking was found to be more superior to foliar spray. Seed soaking of IAA and L-TRP with *Rhizobium* inoculation produced maximum number of nodules pot^{-1} (63, 69), nodule mass (0.462, 0.458 g pot^{-1}), root length (49, 45 cm) and root mass (40.7, 29.8 g pot^{-1}) as compared to *Rhizobium* inoculation alone (40; 0.327 g pot^{-1}; 30 cm and 19.8 g pot^{-1}), respectively.

DISCUSSION

Rhizobium, very well known for its symbiotic relationships with legumes and responsible to fix nitrogen, capable to produce hormones may be considered the most credible mean for plant growth promotion (Mirza *et al.*, 2007; Mehboob *et al.*, 2008). *Rhizobium* inoculations improved germination, nodulation and root/shoot mass of plants (Zahran, 2001; Zahir *et al.*, 2004).

Three isolates of mung bean *Rhizobium* sp. were screened with and without L-TRP for their auxin production potential and other biochemical tests. All isolates of *Rhizobium* sp. produced IAA equivalents without L-TRP and their biosynthesis potential was much enhanced with L-TRP. Auxin biosynthesis potential and different qualitative biochemical tests of different isolates (Table 1). Isolate Mb_3 produced the highest IAA equivalents with and without L-TRP. Microbial biosynthesis of auxins with and without L-TRP was reported by many researchers (Sarwar *et al.*, 1992; Khalid *et al.*, 2001; Zahi r*et al.*, 2004). L-tryptophan is the physiological precursor of auxins act like substrate and microbial biosynthesis of auxins from L-TRP is carried out by two main pathways i.e. tryptophan dependent and tryptophan independent pathways. Under the title of tryptophan dependent pathways, four pathways were proposed viz. i) indole-3-pyruvic acid (IPA), ii) indole-3-acetamide, iii) tryptamine (TAM) pathway, and iv) indole-3-acetaldoxime (IAOX) pathway (Mano and Nemoto, 2012).

Isolate Mb_3 was evaluated for pot studies by introducing IAA and L-TRP @10^{-5} M each with two method of application i.e. seed soaking and foliar spray for the promotion for mung bean growth with and without *Rhizobium* inoculation. Results revealed that IAA and L-TRP either applied as seed soaking or foliar spray promoted the yield, NP content in plant matter, grains and root parameters significantly compared to un-inoculated control. Seed soaking proved better for application of IAA and L-TRP than foliar spray.

Rhizobium inoculation either applied with IAA or L-TRP enhanced the yield attributes and NP contents of mungbean. IAA and L-TRP @ 10^{-5} M influenced the plant growth with and without *Rhizobium* inoculation owed to constant supply PGR's for plants reported by many workers (Khalid *et al.*, 2006). Exogenous application of IAA and L-TRP improved the root architecture and thus promotes the nutrient uptake resulted in better plant growth (Khalid *et al.*, 2006; Glick *et al.*, 2007; Idris *et al.*, 2007). Exogenous application of IAA influenced the plant growth by enlarging leaf area and thus enhanced photosynthetic activities and translocation of carbohydrates in plants. IAA applied exogenously enhanced the root system proliferation, provides more niches for the *Rhizobium* thus promotes nodulation results in better N_2-fixation and uptake of nutrients. Exogenous application of IAA under sub-optimal conditions provides a sufficient source for better growth of plants (Mirza *et al.*, 2007; Zahir *et al.*, 2010). Studies confirmed that exogenous application of IAA improved the growth of plants (Sachdev *et al.*, 2009). Exogenous application of L-TRP provides a continuous source of hormones to plants thus more developed root system, nutrient uptake and thus plant ontogeny (Mirza *et al.*, 2007; Zahir *et al.*, 2010). Bacterial inoculation in the presence of IAA and L-TRP might alter the endogenous hormonal level and influenced the plant shoot/root growth by enhancing meristematic activity of cells, regulation of growth stages of plants ultimately better growth of plants (Khalid *et al.*, 2006**;** Glick *et al.*, 2007; Idris *et al.*, 2007; Sachdev *et al.*, 2009).

Rhizobium inoculation in the presence of IAA and L-TRP exhibited higher NP- contents in plant/grains owing to increase in flourishing root system and nutrient mobilization. *Rhizobium* inoculation with exogenous application of IAA and L-TRP resulted in expansion of root surface area thus more nutrient uptake (Yuming*et al.*, 2003; Naeem *et al.*, 2004). *Rhizobium* inoculation with IAA and L-TRP either applied as seed soaking or foliar spray influenced the yield parameters and nodulation have also been reported by other workers (Garcia *et al.*, 2004; Zahir*et al.*, 2004).

Auxin as (IAA) and L-tryptophan as (L-TRP) with *Rhizobium* inoculation further enhanced the yield and root parameters resulted in enhanced pod, straw yield and NP-content in plant and grains might be due to the increase in root length/mass, thus provide more niches for nodulation. These studies compared the effectiveness of two methods of application i.e. seed soaking or foliar spray for IAA and L-TRP. Seed soaking proved to be better option for both IAA and L-TRP. Fatima *et al.* (2008) reported that application methods of plant hormones showed variable response as seed soaking or foliar spray along with inoculation while Singh (1993) observed that application of growth regulators as foliar spray brought considerable changes in nodulation.

***Conclusion*:** The results clearly demonstrated that application of IAA and L-TRP with *Rhizobium* inoculation enhanced the yield parameters of mungbean, improved nodulation, postharvest soil N, available P and seed soaking proved better than foliar spray. More comprehensive and detailed studies should be carried out to validate the application of growth hormones under field conditions.

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