INFLUENCE OF AZOTOBACTER AND IAA ON SYMBIOTIC PERFORMANCE OF RHIZOBIUM AND YIELD PARAMETERS OF LENTIL

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ABSTRACT

Effect of Azotobacter and indole acetic acid (IAA) on symbiosis and growth parameters of lentil was studied in pots at Soil Bacteriology Section, Ayub Agricultural Research Institute, Faisalabad, Pakistan during the year 2008-09. Inocula were tested on lentil cultivar Masoor-93 at two levels of fertilizers (12-30 and 24-60 kg NP/ha). Data showed that co-inoculation of Rhizobium and Azotobacter significantly excelled in biomass (27.67 g/pot), number of nodules (68.6/plant), nodular mass (1.95 g/plant), root length (39 cm), shoot length (26.3 cm), root weight (7.2 g/pot) and shoot weight (6.8 g/pot) at full dose of fertilizer. Biomass yield with Rhizobium (27.13 g/pot) at full dose of fertilizer was also statistically at par. Chemical analysis of soil depicted that co-inoculation of Rhizobium and Azotobacter at full and half dose of fertilizer provided non-significant but the highest percentage of nitrogen viz. 0.050 and 0.047, respectively. Statistically significant concentration of available phosphorus (16.63 and 15.50 mg/kg) was also observed in co-inoculation at both levels of fertilizers. Chemical analysis of plant matter showed significantly high value of nitrogen (4.4%) due to co-inoculation followed by Rhizobium alone (4.21%) at full dose of fertilizer. Phosphorus percentage of plant matter was statistically non-significant in all treatments but was higher than control. Co-inoculation at full dose of fertilizer showed significantly higher uptake of nitrogen (1.217 g/pot) and phosphorus (0.139 g/pot) by plant than all other treatments. The results further revealed that inoculation of seed by Rhizobium and Azotobacter alone had positive effect on growth and yield parameters but co-inoculation produced better results.

KEYWORDS: Lens culinaris; nitrogen fertilizer; phosphate fertilizer; Azotobacter; Rhizobium; agronomic characters; Pakistan.

INTRODUCTION

Lentil (Lens culinaris) an important pulse crop of sub-continent, is usually grown on poor and marginal land with minimum water requirement. It is a

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good source of protein (35-40%), minerals (30-45%) and vitamins (22%). It is considered as second major pulse crop after chickpea in Pakistan having 29.2 thousand hectares area and 21.1 thousand tons production in the year 2008-09. Average yield of lentil in our country is 646 kg per hectare (1).

*Azotobacter*, a free living microbe, acts as plant growth promoting rhizobacteria (PGPR) in the rhizosphere of almost all crops. Such PGPRs also fix nitrogen for non-legume crops like wheat, cotton, maize and sorghum. These sustain themselves by root exudates and are useful due to the production of growth hormones. Nodulation and yield of several legumes was increased by PGPRs when these were co-inoculated with respective rhizobial symbionts (22, 25).

Plant growth regulators influenced the plant growth when applied in very minute quantity (13). In lower concentration (50mg/kg) indol-3-acetic acid (IAA) optimistically affects the plant growth (15). During the synthesis of carbohydrates it activates sugar translocation (2, 12). Some scientists observed that IAA enhances shoot length (11, 24) accompanied by expansion in shoot diameter while other scientists (16) observed contrast results. However, in both cases it exerts the prolific influence on plant growth.

*Rhizobium* is a microbial symbiont of legumes. In addition to fixing the atmospheric nitrogen through nodulation, it shares many characteristics with other PGPRs including hormones production and solublization of organic and inorganic phosphate (26). *Rhizobium* inoculation gives better results in soils which are low in native *Rhizobium* population (32, 23). *Rhizobium* as seed inoculant increases yield parameters in mung (23).

The aim of present study was to investigate the effect of single and co-inoculation of nitrogen fixing bacteria on important legume lentil as influenced by IAA and *Azotobacter*.

**MATERIALS AND METHODS**

**Microbial cultures**

*Azotobacter*: Isolation of *Azotobacter* strains was carried out in the laboratory of Soil Bacteriology Section, Ayub Agricultural Research Institute, Faisalabad, Pakistan using Jensen agar medium (18). Rhizosphere soil of lentil was sampled from the permanent plot of the Section. Bacteria were isolated by dilution plate technique. One gram of each soil sample was thoroughly mixed with 99ml of sterile distilled water. Series of dilutions ($10^1$, $10^2$, $10^3$, $10^4$ and $10^5$) of suspension were made and $10^5$ dilution was...
inoculated on Jensen agar media and plates were incubated at 30°C for three days. Then single colonies grown on the medium were selected and sub-cultured for purification. For identification, presumptive tests were carried out following standard methods as outlined in Bergey’s Manual of Systematic Bacteriology (28). The pure culture was identified as Azotobacter chroococcum.

**Rhizobium:** Rhizobium was isolated from nodules of lentil. Pink, healthy and undamaged nodules were selected, immersed in 95 percent ethanol for three minutes, rinsed in sterile water and then by acidified mercuric chloride (0.1% W/V) solution (9). The nodules were rinsed for six times in sterile water. With the help of sanitized forceps nodules were crushed under larger drop of water in a petri dish. The juice of these crushed nodules was immediately transferred to the Congo Red Yeast Manitoile Agar (CYMA) media for growth (4). The rhizobial growth that did not attain the colour of Congo red was picked and re-streaked persistently to obtain pure culture. The purified rhizobial culture (Rhizobium leguminosarum) was stored at 4 ± 2°C on slants and maintained for further experimentation.

**Indole acetic acid (IAA):** IAA (10⁻⁵ M) solution @ 20 ml/10 g seed was applied as seed soaking.

**Pot experiments**

It was a pot study conducted for two consecutive years 2007-08 and 2008-09. Pots contained 16 kg clay loam soil having a pH of 8.2, 1.7dS/m EC, 0.031% nitrogen, 9.2 mg/kg Olsen P and 0.61% soil organic matter. Seed of lentil (cv.Masoor-93) was surface sterilized by 95% ethanol and mercuric chloride, followed by vigorous washing with sterile distilled water. Following ten treatments were tried at two fertilizers levels (12-30 and 24-60 kg NP/ha).

- **T1** = Control-1 (Half dose of NP @ 12-30 kg NP/ha un-inoculated)
- **T2** = Control-2 (Full dose of NP @ 24-60 kg NP/ha un- inoculated)
- **T3** = T1 + *Rhizobium* inoculation
- **T4** = T2 + *Rhizobium* inoculation
- **T5** = T1 + *Azotobacter* inoculation
- **T6** = T2 + *Azotobacter* inoculation
- **T7** = T1 + Co-inoculation of *Rhizobium* & *Azotobacter* in 1:1
- **T8** = T2 + Co-inoculation of *Rhizobium* & *Azotobacter* in 1:1
- **T9** = T1 + IAA
- **T10** = T2 + IAA

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Inoculums were used as seed coating and IAA as seed soaking. Layout was CRD with four repeats. When the crop was blooming flowers, one repeat was uprooted and data regarding nodule number, nodular mass, root length, root weight, shoot length and shoot weight were recorded. After harvest biomass yield, nitrogen and phosphorus contents in plant as well as soil nitrogen and available phosphorus were also recorded. Nitrogen was determined according to Kjeldhal method (19) while phosphorus by modified Olsen method (21). Data of two years were averaged and subjected to statistical analysis, following completely randomized design (8). The differences among treatment means were checked by applying the Duncan’s multiple range tests (15).

RESULTS AND DISCUSSION

Nodulation

The results showed that ability of *Rhizobium* for fixing nitrogen through nodulation was supported by *Azotobacter*. Nodulation was significantly higher (62.6 and 68.6 nodules/plant) in co-inoculation of *Rhizobium* and *Azotobacter* at half (T7) and full dose (T8) of fertilizer, respectively (Fig. 1). It was followed by *Rhizobium* alone i.e. T3 (40 nodules/plant) and T4 (46 nodules/plant) at half and full dose of fertilizers, respectively.

![Fig. 1. Effect of co-inoculation of *Rhizobium*, *Azotobacter* and IAA on number of nodules in lentil.](image-url)
Azotobacter produced growth hormones which resulted in root elongation thus providing more infection sites for nodulation. These results are supported by Chandra and Pareek (6) who observed that Azotobacter increases nodulation and yield of several legume species after co-inoculation with its respective rhizobial symbionts. Similar effect of Rhizobium inoculation has also been reported by other scientists (5, 31) in other legumes. Co-inoculation also gave significantly higher nodular mass (1.51 and 1.59 g/plant) at half (T7) and full dose (T8) of fertilizer, respectively (Fig. 2). Rhizobium inoculation also showed increase in nodular mass (1.39 and 1.45 g/plant) at half (T3) and full dose (T4) of fertilizer, respectively which was statistically at par with co-inoculation at half dose of fertilizer. Nodular mass in Azotobacter treatment and IAA was statistically at par with each other (Fig. 2). The indole-3-acetic acid (IAA) arbitrates a number of processes in plant growth and development including general root and shoot architecture (27). The improved root structure thus helps increase the nodulation.

**Root length and root weight**

Inoculation of lentil with Rhizobium and Azotobacter significantly enhanced the root length and root weight (Fig. 3). Co-inoculation significantly increased the root length (36.7 and 39 cm) at half (T7) and full dose (T8) of fertilizer, respectively compared to their respective controls (25.5 and 27cm). These treatments increased root length by 43.9 and 44.4 percent over control.
Azotobacter chroococcum exerted optimistic effect on plants and did not antagonize the symbiont (7). Increase in root length in \( T_3 \) (Rhizobium at half dose of fertilizer) was statistically at par with \( T_{10} \) (IAA at full dose of fertilizer).

Co-inoculation also improved root weight by 34.61 and 30.91 percent in \( T_7 \) and \( T_8 \), respectively compared to control (Fig. 4, Table 1). The introduced Rhizobium had better colonization than native rhizobia in the rhizosphere of 

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**Fig. 3.** Effect of co-inoculation of Rhizobium, Azotobacter and IAA on root length in lentil.

**Fig. 4.** Effect of co-inoculation of Rhizobium, Azotobacter and IAA on root weight in lentil.
Table 1. Percent increase in yield parameters due to different treatments over control.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Biomass</th>
<th>Root length</th>
<th>Shoot length</th>
<th>Root weight</th>
<th>Shoot weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1=Control</td>
<td>8.6</td>
<td>19.2</td>
<td>7.0</td>
<td>19.23</td>
<td>19.2</td>
</tr>
<tr>
<td>T2=Control</td>
<td>5.7</td>
<td>24.4</td>
<td>4.6</td>
<td>21.82</td>
<td>23.5</td>
</tr>
<tr>
<td>T3</td>
<td>5.0</td>
<td>37.2</td>
<td>20.0</td>
<td>13.46</td>
<td>13.5</td>
</tr>
<tr>
<td>T4</td>
<td>1.22</td>
<td>34.4</td>
<td>12.2</td>
<td>9.09</td>
<td>17.2</td>
</tr>
<tr>
<td>T5</td>
<td>16.5</td>
<td>43.9</td>
<td>27.5</td>
<td>34.61</td>
<td>33.00</td>
</tr>
<tr>
<td>T6</td>
<td>7.89</td>
<td>44.4</td>
<td>11.3</td>
<td>30.91</td>
<td>26.48</td>
</tr>
<tr>
<td>T7</td>
<td>13.63</td>
<td>16.36</td>
<td>23.0</td>
<td>21.20</td>
<td>15.90</td>
</tr>
<tr>
<td>T8</td>
<td>2.7</td>
<td>21.15</td>
<td>5.4</td>
<td>16.40</td>
<td>11.11</td>
</tr>
</tbody>
</table>

legumes and enhanced the root weight by 19.23 and 21.82 percent at half (T3) and full (T4) dose of fertilizer, respectively while 13.46 and 9.09 percent increase in root weight was noted in Azotobacter at same levels of fertilizers over control. IAA increased the root length by 21.20 and 16.40 percent over control at full and half doses of fertilizers (Table 1) which is less than Rhizobium and co-inoculation and more than Azotobacter. Better root and shoot formation was also observed in lemon by Seran and Umadevi (27).

**Shoot length and shoot weight**

*Rhizobium* in symbiosis with legumes (T4) significantly enhanced the shoot length (Fig. 5) and shoot weight (Fig. 6) but co-inoculation of *Rhizobium* and
Azotobacter had also better effect on both these traits. Shoot length increased to 26.3 cm with co-inoculation at full dose of fertilizer (T_8) and was statistically at par with *Rhizobium* (26.6cm) (T_4) compared to control (23.7cm) (Fig. 5). *Azotobacter chroococcum* plays beneficial role in plants alongwith the respective symbiont (6). Increase in shoot length by *Azotobacter* (24.8cm) and IAA (25cm) was statistically at par but significantly higher than control. During the carbohydrates synthesis, IAA activates sugar translocation (2, 12). Some scientists (11, 16) observed that IAA caused increase in shoot length while other reported the decrease, accompanied by expansion in diameter of shoot (13). However, in both cases it exerted the affirmative influence on plant growth.

There was 26.4 percent increase in shoot weight by co-inoculation at full dose of fertilizer (T_8) followed *Rhizobium* (T_4) compared to control (Table 1). Increase in shoot weight by *Azotobacter* was upto 17.2 percent which was at par with IAA.

**Biomass of lentil**

Co-inoculation of lentil with *Rhizobium* and *Azotobacter* significantly enhanced the biomass yield. The highest biomass (27.67 and 27.0 g/pot) (Table 2) was produced by co-inoculation at full (T_7) and half dose (T_8) of fertilizer, respectively which was statistically at par with *Rhizobium* at full dose of fertilizer (27.13 g) (T_4). The results are supported by earlier reports on enhanced biomass due to rhizobial inoculation (29, 34).

Influence of Azotobacter and IAA on lentil yield

Table 2. Effect of co-inoculation and IAA on biomass yield and NP contents of soil and plant.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Biomass yield (g/pot)</th>
<th>N in soil (%)</th>
<th>Available P (mg/kg)</th>
<th>N in plant matter (%)</th>
<th>P in plant matter (%)</th>
<th>N uptake by plant (g/pot)</th>
<th>P uptake by plant (g/pot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 = Control 12-30 kg NP/ha</td>
<td>23.17g</td>
<td>0.033</td>
<td>9.70d</td>
<td>2.98f</td>
<td>0.309i</td>
<td>0.688</td>
<td>0.070</td>
</tr>
<tr>
<td>T2 = Control 24-60 kg NP/ha</td>
<td>25.67bc</td>
<td>0.035</td>
<td>10.90d</td>
<td>3.03f</td>
<td>0.321f</td>
<td>0.777</td>
<td>0.079</td>
</tr>
<tr>
<td>T3 = Control + Rhizobium</td>
<td>25.17bc</td>
<td>0.046</td>
<td>13.13bc</td>
<td>4.00cd</td>
<td>0.436c</td>
<td>0.980</td>
<td>0.105</td>
</tr>
<tr>
<td>T4 = Control-2 + Rhizobium</td>
<td>27.13a</td>
<td>0.047</td>
<td>14.27b</td>
<td>4.21b</td>
<td>0.462b</td>
<td>1.100</td>
<td>0.120</td>
</tr>
<tr>
<td>T5 = Control-1 + Azotobacter</td>
<td>24.33cd</td>
<td>0.042</td>
<td>13.43bc</td>
<td>3.96d</td>
<td>0.399c</td>
<td>0.624</td>
<td>0.097</td>
</tr>
<tr>
<td>T6 = Control-1 + Azotobacter</td>
<td>26.00b</td>
<td>0.043</td>
<td>14.37ab</td>
<td>3.99cd</td>
<td>0.411d</td>
<td>1.077</td>
<td>0.109</td>
</tr>
<tr>
<td>T7 = Control-1 + Co-inoc.</td>
<td>27.00a</td>
<td>0.047</td>
<td>15.50ab</td>
<td>4.10bc</td>
<td>0.501a</td>
<td>1.107</td>
<td>0.129</td>
</tr>
<tr>
<td>T8 = Control-2 + Co-inoc.</td>
<td>27.67a</td>
<td>0.050</td>
<td>16.63a</td>
<td>4.40a</td>
<td>0.503a</td>
<td>1.217</td>
<td>0.139</td>
</tr>
<tr>
<td>T9 = Control-1 + IAA</td>
<td>26.31b</td>
<td>0.036</td>
<td>12.00c</td>
<td>3.26e</td>
<td>0.331g</td>
<td>0.858</td>
<td>0.085</td>
</tr>
<tr>
<td>T10 = Control-2+ IAA</td>
<td>26.37ab</td>
<td>0.037</td>
<td>13.47bc</td>
<td>3.32e</td>
<td>0.356f</td>
<td>0.902</td>
<td>0.089</td>
</tr>
<tr>
<td>LSD</td>
<td>1.337</td>
<td>NS</td>
<td>1.4172</td>
<td>0.1204</td>
<td>0.007424</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Means sharing same letter(s) in a column do not differ significantly (p<0.05).

Increase in biomass with co-inoculation was 7.8 and 16.5 percent compared with control (Table 1). These results agree to those of Seran and Umadevi (27) who stated that Azotobacter chroococcum when applied with symbionts promotes its efficiency (33). Azotobacter (T5 and T6) produced 5.0 and 1.22 percent more biomass than their respective control.

IAA treatment also showed better crop stand and produced 26.33 and 26.37g/pot biomass at half (T9) and full dose (T10) of fertilizer, respectively which was significantly higher than Azotobacter but less than Rhizobium. Biomass increase in IAA treatments was 13.63 and 2.7 percent compared to its respective control showing better performance of co-inoculation and Rhizobium than IAA (Table 1).

Post harvest soil analysis

The data (Table 2) showed that soil nitrogen and available phosphorus was significantly higher by co-inoculation of Rhizobium and Azotobacter treatments as compared to control. Nitrogen percentage was statistically non-significant with all inoculation treatments but higher than control. Maximum available phosphorus contents (16.63 and 15.5 mg/kg) were observed by co-inoculation of Rhizobium and Azotobacter at full (T8) and half (T7) dose of fertilizer which was significantly higher than all other treatments. Co-inoculation showed maximum increase in available phosphorus (56%) compared to control while 31.6 percent increase was observed by Azotobacter inoculation alone (Table 2). Similar increase in results has also been reported previously (23, 34). Rhizobium and Azotobacter produced organic acids which lowered the soil pH and thus resulted in phosphate solubilization. Combined effect of Rhizobium and Azotobacter enhanced the availability of P than all other inoculation treatments.

Plant analysis

The data (Table 2) further showed that co-inoculation (T6) produced significantly higher nitrogen in plant (4.4%) as compared to control (3.03%) followed by *Rhizobium* inoculation (T4) (4.2%) at same level of fertilizer. Phosphorus percentage was significantly higher (0.503) in co-inoculation treatment (T8) followed by T7 (0.501) compared to control (0.321). Nitrogen and phosphorus uptake by plant was statistically non-significant in all treatments. However, percent increase in nitrogen uptake by co-inoculation was 56.6 percent more as compared to control while it was 41.5 percent by *Rhizobium* alone. Co-inoculation contributed maximum uptake of phosphorus (0.139g/pot) with 51.8 percent increase in case of *Rhizobium* inoculation as compared to control. Previous studies (7, 10) showed that *Azotobacter* in co-inoculation contributed more in N-fixation and uptake by facilitating *Rhizobium*. Also combination of *Rhizobium* and *Azotobacter* enhanced the nutrient availability to plants in earlier studies (10).

REFERENCES

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