Influence of Bioinoculants on Growth, Nutrient Uptake and Yield of Green Gram [Vigna radiata (L.) Wilczek]

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Influence of Bioinoculants on Growth, Nutrient Uptake and Yield of Green Gram \([Vigna radiata (L.) Wilczek]\)

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**ABSTRACT.** Green gram \([Vigna radiata (L.) Wilczek]\) seedlings were inoculated either individually with arbuscular mycorrhizal (AM) fungi \([Glomus fasciculatum (Gerd. & Trappe) Walker]\), \(Rhizobium japonicum\), \(Cercospora\) sp. and \(Trichoderma harzianum\) or with various combinations using unsterile soil. The experiment was conducted in a completely randomized block design under nursery conditions. Plants were harvested at 20 days interval up to 60 days after inoculation. Bioinoculation increased plant growth, leaf area, chlorophyll contents, nodule numbers, nodule dry weights, pod numbers and dry weights, plant tissue and soil nutrient depletion. Microbial populations also increased in inoculated soils, which were related to each other. This study clearly indicates that
the effect of bioinoculation in green gram was enhanced, when plants were inoculated with various combinations of microbes compared to individual applications. doi:10.1300/J064v31n03_07 [Article copies available for a fee from The Haworth Document Delivery Service: 1-800-HAWORTH. E-mail address: <docdelivery@haworthpress.com> Website: <http://www.HaworthPress.com> © 2007 by The Haworth Press. All rights reserved.]

KEYWORDS. Bioinoculants, biocontrol agents, green gram, interaction, Vigna radiata

INTRODUCTION

Green gram is an important traditional leguminous crop grown throughout Southern Asia, Central Africa, warmer parts of China and the USA (Janoria et al., 1984). It is a short duration crop and has wide adaptability. It is grown all year round as a pure crop or as an intercrop in double and or multiple cropping systems (Thangavel, 2002). Being a legume it could naturally produce protein enriched food without addition of any synthetic nitrogen fertilizers (Salunkhe, 1982). In general green gram production fluctuates widely with space and time because of several biotic and abiotic constraints under which it is cultivated.

A renewed interest in low-input agriculture has resulted in harnessing the role of soil microorganisms that increase soil fertility or improve plant nutrition and thereby health. Though it is critical to assess the impact of interactions of the microbes among themselves and also on other key rhizosphere processes, several studies have been conducted to test the ability of microbial activities on plant growth performance (Elliott and Lynch, 1995).

Microbial inoculation results in complex interactions between the plants and their associated rhizosphere inhabitants. It is well documented that plant-growth-promoting rhizosphere microorganisms like Pseudomonas, Trichoderma, Azospirillum and Rhizobium diazotrophs enhance arbuscular mycorrhizae (AM) fungal colonization and activity resulting in better plant performance (Subba Rao, 1985; Paula et al., 1992; Biro et al., 1993; Garbaye, 1994; Barea et al., 1998; Hazarika et al., 2000; Mar Vazquez et al., 2000; Tsimilli-Michael et al., 2000; Muthukumar et al., 2001; Rudresh et al., 2005). Similarly, biological control preserves environmental quality by reducing chemical inputs in future sustainable agricultural management practices (Altieri, 1994; Barea and Jeffries, 1995).
The positive influence of *Rhizobium* (Emmimath, 1984; Kulkarni et al., 1984; Nambiar et al., 1984) and AM fungi on the growth and development of green gram (Kucey and Paul, 1982; Manjunath and Bagyaraj, 1984) has been well documented (Middleton et al., 1989). However, the complexity and the outcome of the microbial interactions tend to increased with increasing the number of microorganisms involved. Such studies on the interactions of bioinoculants (AM fungi, *Rhizobium*) and the biocontrol agent *Trichoderma* in the rhizosphere of green gram under a particular suit of environmental conditions are lacking. The aim of the present study was to evaluate and assess interactive effects of bioinoculants and a biocontrol agent on growth, nutrient uptake, yield and suppression of “spot” disease in green gram.

**MATERIALS AND METHODS**

**Experimental Design**

The experiment was carried out in the nursery of the Botany Department, Bharathiar University, Coimbatore, Tamil Nadu, India. Green gram seedlings were inoculated with the biocontrol agent (*Trichoderma*), AM fungi, N₂-fixing bacteria (*Rhizobium japonicum*) and a pathogen (*Cercospora* sp.) either individually or in various combinations and the uninoculated seedlings served as control. Seedlings were raised in polybags (23 × 10 cm²; 3 replicates) and arranged in a completely randomized block design. Plants were watered as when necessary throughout the duration of the experiment. The positions of the polybags were altered once in every 15 days to expose plants to uniform conditions.

**Substrate and Sowing**

Green gram [*Vigna radiata* (L.) Wilczek cv. ‘KM2’] seeds were obtained from Seed Bank of Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The seeds were directly sown in polybags after microbial inoculations according to treatments. The polybags were filled with ca 3 kg of sand:red soil:cow dung (1:2:1) mix. The soil had 7.8 pH, 42.35 mS cm⁻¹ electrical conductivity, 0.14 mg kg⁻¹ total N, 0.016 mg kg⁻¹ of available P and 0.12 mg kg⁻¹ exchangeable K prior to cow dung amendment. The indigenous AM fungi, *Rhizobium* and *Trichoderma* populations were 8.84 propagules, 0.23 and 2.37 colony forming unit (CFU) g⁻¹ of soil, respectively.
Inoculum

AM Fungi

AM fungal inoculum at the rate of 10,000 propagules of a single or equal proportion (5,000 each) of two species was placed before sowing the seeds. Experimental plants not inoculated with AM fungi received the same amount of sterile inoculum, which had been consecutively autoclaved for three times at 121°C for 90 min. at regular intervals. Soil microbes in AM fungal inoculum were equalized across treatments by applying 25 ml “microbial wash” to each bag. This “microbial wash” was prepared by blending 100 g of soil containing AM fungal inocula in 1,000 ml deionized water and filtering it three times through a 25-μm sieve. AM fungal inoculum consisted of soil and root containing spore from a pot culture of sorghum (Sorghum vulgare Monech.), which was colonized by Glomus fasciculatum (Gerd. & Trappe) Walker and grown for 9 months. Glomus fasciculatum originating from the rhizosphere of green gram was multiplied in pot cultures using sorghum as host.

Rhizobium japonicum

Inoculum of Rhizobium sp. was obtained from the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. Five grams of charcoal-based bacterial inoculum (10^9 CFU g⁻¹) was prepared and placed as a thin layer just 2 cm below the soil surface in each bag before sowing the seeds.

Cercospora sp.

Cercospora sp. was obtained from the Department of Agricultural Pathology, Tamil Nadu Agricultural University, Coimbatore. Five millilitres of liquid-based fungal inoculum (80 × 10^3 CFU g⁻¹) was sprayed on the leaf surface of each plant after 7 days of seedling emergence.

Trichoderma harzianum

Trichoderma harzianum was obtained from the Department of Biocontrol, Tamil Nadu Agricultural University, Coimbatore. Five grams of
peat-based fungal inoculum \((80 \times 10^3 \text{ CFU g}^{-1})\) was spread as a thin layer just 2 cm below the soil surface of each bag before sowing the seeds.

**Treatments**

Various combinations of inocula were prepared as indicated below to test their interactions and influence on plant growth and disease incidence reduction. The combinations used were: (1) Control; (2) *Glomus fasciculatum*; (3) *Rhizobium japonicum*; (4) *Cercospora* sp.; (5) *Trichoderma harzianum*; (6) *Glomus fasciculatum + Rhizobium japonicum*; (7) *Glomus fasciculatum + Cercospora* sp.; (8) *Glomus fasciculatum + Trichoderma harzianum*; (9) *Glomus fasciculatum + Rhizobium japonicum + Cercospora* sp.; (10) *Glomus fasciculatum + Rhizobium japonicum + Trichoderma harzianum*; (11) *Glomus fasciculatum + Cercospora* sp. + *Trichoderma harzianum*; (12) *Rhizobium japonicum + Cercospora* sp. + *Trichoderma harzianum*; and (13) *Glomus fasciculatum + Rhizobium japonicum + Cercospora* sp. + *Trichoderma harzianum*.

**Harvest and Measurements**

Green gram plants were harvested once in 20 days up to 60 days after inoculation (DAI) with almost their entire root system. Triplicates were taken for analysis. However for brevity, data of final harvest (60 DAI) only are presented. During the harvest, plant growth parameters such as shoot and root lengths, shoot and root dry weights, nodule and pod numbers and their dry weights, and numbers of branches were recorded. After harvest, a weighted portion of the root sample was fixed in formalin acidic acid (FAA) for the assessment of AM fungal colonization. Leaf area was measured using the LI–3000 portable leaf area meter (Li–Cov, USA) and leaf chlorophyll content was estimated according to Yoshida et al. (1971).

**Quantitative Estimation of the Microorganisms**

Soil samples were collected in polythene bags and brought to the laboratory. Samples were shade dried and stored at 4°C until further analysis. A dilution plate method was employed for the enumeration of soil microbial populations. Appropriate dilutions and media were chosen...
for respective organism namely, $10^{-3}$ for fungus (potato dextrose agar), and $10^{-5}$ for Rhizobium (Yeast extract agar, Subba Rao, 1986).

**Preparation of Roots and AM Assessment**

Fixed roots were thoroughly washed free of FAA using tap water and were observed under a dissection microscope (20×) for intact AM fungal spores on them. After examination, the roots were cut into 1 cm bits, cleared in 2.5% KOH (Koske and Gemma, 1989), acidified with 5 N HCl and stained with trypan blue (0.5% in lactoglycerol), in which the roots were kept immersed overnight. The stained roots were examined with a compound microscope (200-400×) for AM fungal structures and the percentage of root length colonization was estimated according to the magnified intersection method (McGonigle et al., 1990).

**Enumeration and Identification of AM Fungal Spores**

AM fungal spores in the rhizosphere soil samples were estimated by a wet-sieving and decanting technique of Gerdemann and Nicolson (1963). One hundred grams of soil was dispersed in 1 L water and the suspension was decanted through 710-38-μm sieves. The residues in the sieves were washed into beakers. The sievates were dispersed in water and filtered through gridded filter papers. Each filter paper was placed on a petri dish and scanned under a dissection microscope at 40× magnification and all intact spores were counted. Sporocarps and spore clusters were considered as one unit. Intact AM fungal spores were transferred using a wet needle to polyvinyl alcohol-lactoglycerol with or without Melzer’s reagent on a glass slide for identification. Spores were identified based on spore morphology and sub-cellular characters and compared with original descriptions (Schenck and Perez, 1990). Spore morphology was also compared with the culture database established by INVAM (http://invam.caf.wvu.edu).

**Disease Severity Assessment**

Dimond et al. (1952), grading was used to evaluate the disease (spot disease) severity that was assessed by grading the leaves [0 = No disease (spot) symptom; 1 = 1-10%, slight; 2 = 11-25%, moderate; 3 = 26-50%, heavy; 4 = 51% above, partial dried].
Analysis of Soil Nutrients

Total N and available P were determined, respectively by micro-Kjeldahl and molybdenum blue methods of Jackson (1973). Exchangeable K was extracted from the soil in ammonium acetate solution (pH 7) and estimated using a digital flame photometer (Jackson, 1973).

Analysis of Plant Tissue Nutrients

The plant samples were oven dried and ground to make a fine powder in Willy ball mill and used for the estimation of tissue nutrients. One hundred mg of tissue samples were wet digested in 2 ml of conc. H₂SO₄ containing a catalyst (CuSeO₃). The digested samples were made up to 50 ml and N content in the extract was estimated by micro-Kjeldahl method. Two hundred milligrams of plant tissue was wet digested in 10 ml of a triple acid mixture (nitric, sulphuric and perchloric acid mixture; 9:2:1). The digested samples were made up to 100 ml for P estimation. Phosphorus was estimated colorimetrically following the vanadomolybdate method (Jackson, 1973). Potassium content in the aliquot of the triple acid extract was estimated by emission spectrophotometry using EEL flame photometer (Jackson, 1973).

Nutrient-Use Efficiency

Nutrient-use efficiencies (NUE) were calculated as unit of biomass produced per unit of nutrient content (Marschner, 1995) using the formula,

\[ \text{NUE} = \frac{\text{Plant dry weight (g) (shoot + root)}}{\text{Tissue nutrient content (g) (shoot + root)}} \]

Statistical Analysis

The data were statistically analysed by Analysis of Variance (ANOVA) and means were separated using Duncan’s Multiple Range Test (DMRT). Pearson’s correlation analysis was used to assess the relationships between plant biomass, microbial population and plant tissue and soil nutrients (Zar, 1984). Data on AM colonization were arcsine transformed and AM fungal spore number were log transformed prior to analysis.
RESULTS

Plant Growth and Biomass

Green gram plants exhibited various responses to inoculation with different microbes and their combinations (Table 1). Generally inoculated plants were taller, had longer tap roots and more number of leaves with large surface area. Shoot and root dry weights, nodule numbers and dry weights were also higher compared to uninoculated control. Combined inoculation of *G. fasciculatum*, *R. japonicum* and *T. harzianum* increased plant height, tap root length, shoot and root dry weight by 2-fold over uninoculated control and 3-fold over those inoculated with *Cercospora* sp. alone. However, leaf numbers and area, nodule number and their dry weights were higher in plants inoculated with *G. fasciculatum*, *R. japonicum*, *Cercospora* sp. and *T. harzianum* at 60 DAI. Generally, plants inoculated with *Cercospora* sp. alone had reduction in their growth and biomass.

Disease Incidence

Disease incidence in green gram significantly varied between pathogen inoculated and uninoculated plants. Disease severity was found to be 2-, 3- and 3-fold lower in plants inoculated with *G. fasciculatum*, *R. japonicum* and *T. harzianum*, respectively, than in uninoculated plants and 3, 3 and 4 times lower than *Cercospora* sp. inoculated plants during 20 (F12, 26 = 76.52; P < 0.01), 40 (F12, 26 = 91.70; P < 0.01) and 60 (F12, 26 = 46.53; P < 0.01) DAI, respectively (Table 2). Least disease severity was noticed in plants inoculated with the combination of *G. fasciculatum*, *R. japonicum* and *T. harzianum*.

Pod Numbers and Dry Weights

Pod numbers and their dry weights of green gram increased in plants which had combined inoculation of *G. fasciculatum*, *R. japonicum* and *T. harzianum* and they produced flowers at 40 DAI. As shown in Table 2, number of pods and dry weights increased between 2- and 5-fold over uninoculated control. Generally, individual inoculation with *Cercospora* sp. reduced pod numbers and their dry weights.
TABLE 1. Effect of bioinoculants and biocontrol agent on the growth of *Vigna radiata*.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Shoot height (cm plant$^{-1}$)</th>
<th>Tap root length (cm plant$^{-1}$)</th>
<th>Leaf number (plant$^{-1}$)</th>
<th>Leaf area (cm$^2$ plant$^{-1}$)</th>
<th>Shoot dry weight (g plant$^{-1}$)</th>
<th>Root dry weight (g plant$^{-1}$)</th>
<th>Root/ shoot ratio</th>
<th>Nodule number (plant$^{-1}$)</th>
<th>Nodule dry weight (g plant$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.10a</td>
<td>18.47d</td>
<td>15.70a</td>
<td>48.43b</td>
<td>1.23a</td>
<td>0.15ab</td>
<td>0.116e</td>
<td>16.0b</td>
<td>0.032b</td>
</tr>
<tr>
<td>G.f</td>
<td>20.57ab</td>
<td>24.37f</td>
<td>24.00b</td>
<td>63.33c</td>
<td>1.43b</td>
<td>0.21de</td>
<td>0.145g</td>
<td>19.0c</td>
<td>0.036c</td>
</tr>
<tr>
<td>R</td>
<td>24.80ab</td>
<td>21.50e</td>
<td>38.00e</td>
<td>84.37f</td>
<td>1.55cd</td>
<td>0.19cd</td>
<td>0.126f</td>
<td>26.0d</td>
<td>0.039d</td>
</tr>
<tr>
<td>C</td>
<td>14.53a</td>
<td>11.80a</td>
<td>14.70a</td>
<td>38.57a</td>
<td>1.18a</td>
<td>0.13a</td>
<td>0.103cd</td>
<td>11.7a</td>
<td>0.028a</td>
</tr>
<tr>
<td>T.h</td>
<td>21.43ab</td>
<td>24.23f</td>
<td>30.70c</td>
<td>74.37d</td>
<td>1.47bc</td>
<td>0.23ef</td>
<td>0.143g</td>
<td>16.7b</td>
<td>0.034bc</td>
</tr>
<tr>
<td>G.f + R</td>
<td>36.43cd</td>
<td>32.37g</td>
<td>52.30f</td>
<td>98.30g</td>
<td>2.76e</td>
<td>0.25fg</td>
<td>0.097ab</td>
<td>37.7f</td>
<td>0.077f</td>
</tr>
<tr>
<td>G.f + C</td>
<td>19.60ab</td>
<td>16.53b</td>
<td>34.00d</td>
<td>82.47e</td>
<td>1.64d</td>
<td>0.17bc</td>
<td>0.115e</td>
<td>32.3e</td>
<td>0.066e</td>
</tr>
<tr>
<td>G.f + T.h</td>
<td>18.60ab</td>
<td>17.50c</td>
<td>35.70de</td>
<td>82.43e</td>
<td>1.46bc</td>
<td>0.19cd</td>
<td>0.125f</td>
<td>33.7e</td>
<td>0.068c</td>
</tr>
<tr>
<td>G.f + R + C</td>
<td>39.57cd</td>
<td>34.53h</td>
<td>53.30fg</td>
<td>105.07h</td>
<td>2.90f</td>
<td>0.27g</td>
<td>0.100abc</td>
<td>37.3f</td>
<td>0.083gh</td>
</tr>
<tr>
<td>G.f + R + T.h</td>
<td>44.33d</td>
<td>38.47i</td>
<td>55.70gh</td>
<td>106.73i</td>
<td>3.26h</td>
<td>0.33h</td>
<td>0.102bc</td>
<td>41.0g</td>
<td>0.085hi</td>
</tr>
<tr>
<td>G.f + C + T.h</td>
<td>41.53d</td>
<td>35.20i</td>
<td>54.30fg</td>
<td>106.60i</td>
<td>3.16gh</td>
<td>0.32h</td>
<td>0.098abc</td>
<td>41.0g</td>
<td>0.082g</td>
</tr>
<tr>
<td>R + C + T.h</td>
<td>40.27cd</td>
<td>35.40i</td>
<td>56.70h</td>
<td>107.50i</td>
<td>3.06g</td>
<td>0.33h</td>
<td>0.108d</td>
<td>43.0g</td>
<td>0.087i</td>
</tr>
<tr>
<td>G.f + R + C + T.h</td>
<td>29.62bc</td>
<td>38.30i</td>
<td>53.70fg</td>
<td>105.47h</td>
<td>3.09g</td>
<td>0.31h</td>
<td>0.096a</td>
<td>41.0g</td>
<td>0.086i</td>
</tr>
</tbody>
</table>

Means followed by a common letter(s) are not significantly different at 5% level according to DMRT.

G.f.: *Glomus fasciculatum*; R: *Rhizobium* sp.; C: *Cercospora* sp.; T.h.: *Trichoderma harzianum*. 
Green gram inoculated with a combination of *G. fasciculatum*, *Rhizobium* sp. and *T. harzianum* had a 3-fold higher chlorophyll *a* content compared to uninoculated plants (Figure 1). In contrast, plants inoculated with *R. japonicum*, *Cercospora* sp. and *T. harzianum* had maximum chlorophyll *b* and total chlorophyll contents. There were significant variations between treatments in chlorophyll *a* ($F_{13, 26} = 16006.11; P < 0.01$), chlorophyll *b* ($F_{13, 26} = 9101.18; P < 0.01$) and total chlorophyll ($F_{13, 26} = 7200.74; P < 0.01$). Generally, plants inoculated with *Cercospora* sp. alone had less chlorophyll *a*, *b* and total chlorophyll contents.

### AM Fungal Colonization and Spore Numbers

Inoculation of *G. fasciculatum*, *R. japonicum* and *T. harzianum* increased AM fungal colonization structures that had developed 20, 8 and 3

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**Plant Chlorophyll Contents**

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**TABLE 2. Effect of bioinoculants and biocontrol agent on disease incidence, pod number and pod dry weight of *Vigna radiata.***

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Disease Incidence (plant$^{-1}$)</th>
<th>Pod Number (plant$^{-1}$)</th>
<th>Pod Dry Weight (plant$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 DAI</td>
<td>40 DAI</td>
<td>60 DAI</td>
</tr>
<tr>
<td>Control</td>
<td>16.0$^{ef}$</td>
<td>18.7$^{g}$</td>
<td>23.3$^{f}$</td>
</tr>
<tr>
<td>G.f</td>
<td>12.0$^{d}$</td>
<td>13.3$^{ef}$</td>
<td>15.0$^{de}$</td>
</tr>
<tr>
<td>R</td>
<td>15.0$^{e}$</td>
<td>17.3$^{g}$</td>
<td>18.0$^{e}$</td>
</tr>
<tr>
<td>C</td>
<td>21.3$^{g}$</td>
<td>22.0$^{h}$</td>
<td>29.0$^{g}$</td>
</tr>
<tr>
<td>T.h</td>
<td>13.0$^{d}$</td>
<td>14.0$^{f}$</td>
<td>17.0$^{e}$</td>
</tr>
<tr>
<td>G.f + R</td>
<td>10.0$^{c}$</td>
<td>12.0$^{de}$</td>
<td>13.0$^{cd}$</td>
</tr>
<tr>
<td>G.f + C</td>
<td>17.0$^{f}$</td>
<td>17.0$^{g}$</td>
<td>18.0$^{e}$</td>
</tr>
<tr>
<td>G.f + T.h</td>
<td>20.0$^{g}$</td>
<td>25.0$^{l}$</td>
<td>25.7$^{l}$</td>
</tr>
<tr>
<td>G.f + R + C</td>
<td>10.0$^{C}$</td>
<td>11.0$^{cd}$</td>
<td>12.3$^{bcd}$</td>
</tr>
<tr>
<td>G.f + R + T.h</td>
<td>7.0$^{A}$</td>
<td>7.0$^{a}$</td>
<td>8.0$^{a}$</td>
</tr>
<tr>
<td>G.f + C + T.h</td>
<td>8.0$^{AB}$</td>
<td>9.7$^{bc}$</td>
<td>11.3$^{bc}$</td>
</tr>
<tr>
<td>R + C + T.h</td>
<td>9.0$^{BC}$</td>
<td>9.0$^{b}$</td>
<td>7.7$^{ab}$</td>
</tr>
<tr>
<td>G.f + R + C + T.h</td>
<td>7.3$^{AB}$</td>
<td>8.0$^{ab}$</td>
<td>9.7$^{ab}$</td>
</tr>
</tbody>
</table>

Means followed by a common letter(s) are not significantly different at 5% level according to DMRT.

G.f.: *Glomus fasciculatum*; R: *Rhizobium* sp.; C: *Cercospora* sp; T.h.: *Trichoderma harzianum*
FIGURE 1. Effect of bioinoculants and biocontrol agent on chlorophyll $a$, $b$ and total chlorophyll content of green gram at 60 days after inoculation (DAI). Bar bearing the same letters are not significantly different at 5% level according to DMRT.
times higher percentage of root length with arbuscules, vesicles and total colonization respectively (Figure 2) compared to uninoculated control. Significant variation was observed between root length with arbuscules ($F_{13, 26} = 2866.64; P < 0.01$), vesicles ($F_{13, 26} = 7158.75; P < 0.01$) and total colonization ($F_{13, 26} = 10606.54; P < 0.01$).

**Population of Bioinoculants**

Inoculation of bioinoculants increased the population of *G. fasciculatum* ($F_{13, 26} = 274.41; P < 0.01$), *R. japonicum* ($F_{13, 26} = 307.46; P < 0.01$) and *Trichoderma harzianum* ($F_{13, 26} = 44.50; P < 0.01$) (Figure 3). However, microbial population of *Cercospora* sp. in inoculated soil was lower and it never exceeded the levels of population observed in uninoculated soils. Populations of these microbes were significantly higher in soils inoculated with a combination of *G. fasciculatum, R. japonicum* and *T. harzianum* compared to other treatments.

**Nutrient Contents and Its Use Efficiency**

Generally, soil (Figure 4) and tissue (Figure 5) nutrient concentrations and nutrient-use efficiency (Figure 6) were significantly higher invariably in all treatments involving microbial combinations at 60 DAI. Plants inoculated with *R. japonicum, Cercospora* sp. and *T. harzianum* had maximum soil N ($F_{13, 26} = 34.16; P < 0.01$) and P ($F_{13, 26} = 27.48; P < 0.01$). Plants inoculated with *G. fasciculatum, R. japonicum* and *T. harzianum* had maximum tissue N ($F_{13, 26} = 183.21; P < 0.01$) and P ($F_{13, 26} = 39.61; P < 0.01$). However, inoculation with *G. fasciculatum, Cercospora* sp. and *T. harzianum* had maximum soil and tissue K ($F_{13, 26} = 15.32$ and $2567.66; P < 0.01$) compared to other treatments. Nitrogen-use efficiency was found to be least in the plants that inoculated with *G. fasciculatum* and *Cercospora* sp. and maximum N-use efficiency ($F_{13, 26} = 924.99; P < 0.01$) was recorded in the plants that were inoculated with *G. fasciculatum, R. japonicum, Cercospora* sp. and *T. harzianum*. Phosphorus-($F_{13, 26} = 3830.38; P < 0.01$) and Potassium-($F_{13, 26} = 47183.73; P < 0.01$) use efficiency were least in the plants which were inoculated with *G. fasciculatum* and *T. harzianum* and maximum use efficiency of P and K were recorded in the plants which had the combinations of *G. fasciculatum, R. japonicum* and *T. harzianum* compared to other treatments.
FIGURE 2. Effect of bioinoculants and biocontrol agent on percent root length with arbuscule (RLA), vesicle (RLV) and total root length colonization (RLC) in green gram at 60 days after inoculation (DAI). Bar bearing the same letters are not significantly different at 5% level according to DMRT.
FIGURE 3. Effect of bioinoculants and biocontrol agent on AM fungal spore number, *Rhizobium* and *Trichoderma* populations in the rhizosphere of green gram under nursery condition at 60 days after inoculation (DAI). Bar bearing the same letters are not significantly different at 5% level according to DMRT.
FIGURE 4. Effect of bioinoculants and biocontrol agent on soil nitrogen, phosphorus and potassium contents in the rhizosphere of green gram under nursery condition at 60 days after inoculation (DAI). Bar bearing the same letters are not significantly different at 5% level according to DMRT.
FIGURE 5. Effect of bioinoculants and biocontrol agent on tissue nitrogen, phosphorus and potassium contents of green gram at 60 days after inoculation (DAI). Bar bearing the same letters are not significantly different at 5% level according to DMRT.

C-Control; T1-G.f; T2-R; T3-C; T4-T; T5-G.f + R; T6-G.f + C; T7-G.f + T;
T8-G.f + R + C; T9-G.f + R + T; T10-G.f + C + T; T11-R + C + T; T12-G.f + R + C + T
FIGURE 6. Effect of bioinoculants and biocontrol agent on nitrogen-, phosphorus- and potassium-use efficiency of green gram at 60 days after inoculation (DAI). Bar bearing the same letters are not significantly different at 5% level according to DMRT.
DISCUSSION

In general plant growth and biomass significantly increased with multiple microbial inoculations. These findings generally agree with earlier studies, where simultaneous inoculation of different microorganisms increased plant growth (Wong and Stenberg, 1979; Hazarika et al., 2000; Mar Vazquez et al., 2000; Tsimilli-Michael et al., 2000; Muthukumar et al., 2001; Rudresh et al., 2005). Plant growth promotion by rhizobacteria and the influence of growth, nodulation and N$_2$-fixation in leguminous and actinorhizal plants by AM fungi were well documented (Russo, 1989; Isopi et al., 1994; Osundina, 1998). It had been also reported that increased cytokinin activity in the shoots in response to mycorrhizal colonization could promote leaf growth through increased cell division and cell expansion (Bass and Kupier, 1989).

Combined inoculation of *G. fasciculatum* along with *R. japonicum* had a significant positive effect on the growth of *V. radiata*. Similar results were observed in *Trifolium alexandrinum*, *T. subterraneum*, *Medicago sativa*, *Glycine max* and *V. radiata*, when inoculated with different AM fungi (Smith and Daft, 1977; Smith et al., 1979; Asimi et al., 1980; Patterson et al., 1990; Young et al., 1988; Hazarika et al., 2000). Rhizobial nodulation was significantly and positively correlated to AM fungal spore number ($r = 0.858; P < 0.01; n = 12$). The improved plant P levels in the inoculated plants with response to higher percent age of root length colonization could be attributed to abundance of nodulation in the host, since nodulation and nitrogen fixation by the bacterial symbiont requires a high level of P in the host tissue (Barea and Azcon-Aguilar, 1983). Plants inoculated with *G. fasciculatum* and *R. japonicum* had fewer but heavier nodules, which is in accordance with the studies of Patterson et al. (1990), Muthukumar and Udaian (1995) and Hazarika et al. (2000), where AM fungal inoculations tend to reduce nodulation but increase nodular biomass in different leguminous hosts. Patterson et al. (1990) indicated that AM fungi affected the nodule development in the lower parts of the roots in *M. sativa* and *T. alexandrinum* due to indirect response of the host plants. Increased P status of the host due to AM fungal association could increase the host’s immunity. This could limit rhizobial infection resulting in the reduction of nodule numbers (Barea and Azcon-Aguilar, 1983). Rhizobial population in the soil was strongly correlated to nodule numbers ($r = 0.845; P < 0.01; n = 12$) and nodule dry weights ($r = 0.804; P < 0.01; n = 12$).
In the present study, microbial populations (AM fungi, *Rhizobium*, and *Trichoderma*) recorded in the rhizospheres of the inoculated plants were positively correlated with each other. Most of the studies indicate that the establishment and functions of many microorganisms in the rhizosphere may not only influence plant growth and development but also influence the co-occurring microbial members of the soil community (Lynch, 1990; Klopper et al., 1991; Hazarika et al., 2000; Mar Vazquez et al., 2000; Tsimilli-Michael et al., 2000; Muthukumar et al., 2001; Rudresh et al., 2005). Specialized activities such as the production of vitamins, amino acids, hormones, etc., may be operating in microbe-microbe interactions involving AM fungi and other soil microorganisms.

In the present study, inoculation of *Rhizobium* sp. was able to improve mycorrhization as shown by other rhizosphere microorganisms (Azcon-Aguilar and Barea, 1992; Barea et al., 1998). In addition, percentage of root length arbuscules (RLA) as strongly correlated with *Rhizobium* (*r* = 0.811; *P* < 0.01; *n* = 12) and *Trichoderma* (*r* = 0.679; *P* < 0.01; *n* = 12) populations. However, the precise mechanisms that account for such microbial stimulation of AM fungal formation is unknown. It had been reported that mycorrhization substantially increased the phosphatase activity in marigold roots (Tarafdar and Marschner, 1995). Tien et al. (1979) reported the production of plant hormone-like substances by *Azospirillum*. It was well documented that the AM fungal colonization and its activity were enhanced in the hosts by both *Azospirillum* and *Rhizobium* diazotrophs (Azcon et al., 1978; Sumner, 1990; Paula et al., 1992; Biro et al., 1993; Garbaye, 1994).

Soil microbial growth and its activity were usually limited by the availability of carbon source in the inhabited places, particularly in the rhizospheres (Wardle, 1992; Lynch, 1990). The composition and mass of microorganisms present in the rhizosphere of *V. radiata* were limited by the changes in the quality and quantity of compounds exuded by plants under different treatments. Plant growth, photosynthesis and exudation in a well-established environment could efficiently involve an enormous quantity of carbon (Andrade et al., 1997; Muthukumar et al., 2001). Furthermore, the nutrient status of plants was also known to influence root exudates composition (Kraffeyzky et al., 1984; Lipton et al., 1987).

Several studies revealed that *Trichoderma* could influence plant growth. It was evident in the present study, where the presence of *Trichoderma* was significantly correlated (*P* < 0.01; *n* = 13) to shoot height (*r* = 0.874), root length (*r* = 0.828), leaf area (*r* = 0.837), leaf numbers (*r* = 0.858), shoot dry weights (*r* = 0.823) and root dry weights (*r* = 0.901). The influence of *Trichoderma* includes the production of growth...
hormones (Windham et al., 1986; Chet, 1987; Kleifeld and Chet, 1992; Duffy et al., 1996), solubilization of insoluble major and minor nutrients in the soil (Altomare et al., 1999; Yedidia et al., 2001) and increased uptake and translocation of low-available minerals (Baker, 1989; Kleifeld and Chet, 1992; Inbar et al., 1994; Whipps, 2001). Combined inoculation of *G. fasciculatum* and *T. harzianum* significantly reduced the spore and hyphae density of *Cercospora* sp., which is responsible for spot disease in green gram. However, the population of *Trichoderma* was negatively correlated to spore numbers of *Cercospora* sp. (*r* = 0.799; *p* < 0.01; *n* = 13). Mycorrhizal fungi, *Trichoderma* and *Pseudomonas* strains are well-known combinations to control most of the soil/air/root-borne pathogens. These could have helped the plants indirectly to escape from the incidence of diseases caused by pathogens, due to the production of antimicrobial metabolites in the host (Paulitz and Linderman, 1991). Antibiotic production by *Trichoderma* sp. is a well-documented phenomenon and the reason for its inclusion in biocontrol agents (Calvet et al., 1992; Barea et al., 1998; Sivasithamparam and Ghisalberti, 1998; Howell, 1998, 1999; Hazarika et al., 2000; Mar Vazquez et al., 2000; Tsimilli-Michael et al., 2000; Rudresh et al., 2005).

In the present study the *Trichoderma* population was significantly higher when it was co-inoculated with AM fungi. This observation contradicts those of Green et al. (1999), where the AM fungus *G. intraradices* reduced the population of *T. harzianum*. In addition, *Trichoderma* enhanced root colonization developed by *G. fasciculatum*, which was significantly and positively correlated to %RLA in the present study (*r* = 0.679; *P* < 0.01; *n* = 13). This finding contradicts the observation of Green et al. (1999) where inoculation of *T. harzianum* had been reported to have a negative influence on root colonization developed by *G. intraradices* in cucumber (*Cucumis sativus* L.) (Green et al., 1999).

The uptake and transport of soil N by AM fungal hyphae is well documented (George et al., 1995). The N, P and K contents of dually inoculated plants (*Glomus* + *Rhizobium*) were higher than in plants inoculated with *Rhizobium* alone, suggesting a better N, P and K nutritional status brought about by AM fungi. However, the positive correlation of soil N (*r* = 0.719; *p* < 0.01; *n* = 13), soil P (*r* = 0.902; *p* < 0.01; *n* = 13) and plant tissue N (*r* = 0.833; *p* < 0.01; *n* = 13), tissue P (*r* = 0.824; *p* < 0.01; *n* = 13) and tissue K (*r* = 0.723; *p* < 0.01; *n* = 13) and plant tissue N with %RLA and %RLA positively correlated to N-use efficiency (*r* = 0.603; *p* < 0.01; *n* = 13) and K-use efficiency (*r* = 0.724; *p* < 0.01; *n* = 13), clearly suggested that AM fungi could aid *V. radiata* in the uptake of N directly from the soil.
Green gram inoculated either individually with AM fungi, *Rhizobium* sp. and *T. harzianum* or with their mixtures substantially increased plant growth, nutrient contents and also reduced disease incidence. These responses were either marginal or reached up to several fold when inoculated plants were compared with uninoculated plants. It is clearly indicated that the inoculation of *Cercospora* sp. could affect the growth and uptake of nutrients in *V. radiata* plant growth and nutrient uptake. Inoculation of green gram with a mixture of bioinoculants and biocontrol agent gained higher vigor in the nursery and had the better chances of survival and yield in the field conditions.

**REFERENCES**


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