Efficacy of rhizobial and phosphate-solubilizing bacteria and arbuscular mycorrhizal fungi to ameliorate shade response on six pulse crops

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Efficacy of rhizobial and phosphate-solubilizing bacteria and arbuscular mycorrhizal fungi to ameliorate shade response on six pulse crops

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Abstract Beneficial effects of bio-inoculants on growth and yield of plants grown in sunlight have been reported world over but information on their effect under shade is meagre. Therefore, to assess the effect of shade on bio-inoculants, viz. rhizobial (RB) and phosphate-solubilizing bacteria (PSB), and arbuscular mycorrhizal fungi (AMF), which are associated with intercrops in agroforestry systems, a study was carried out on important rainy (*Glycine max*, *Phaseolus mungo*, and *Vigna radiata*) and winter season pulses (*Cicer arietinum*, *Lens culinaris*, and *Pisum sativum*) under 25% (shade) and 100% (no shade) full sun light. The results showed that plant height was higher under the shade in *G. max*, *P. mungo*, *L. culinaris*, and *P. sativum,* and lower in *V. radiata* and *C. arietinum*. Dry weight and yield plant^{-1} were lower under the shade than the corresponding values in the open for all pulses. In general, bio-inoculants increased plant height, dry weight, and yield plant^{-1} in all pulses, barring a few exceptions. The efficiencies of bio-inoculants in terms of percent increase of yield over respective control were more or less comparable under shade and no shade for most pulses. The shade reduced rhizobial nodulation and AMF colonization in all crops with a few exceptions. Application of bio-inoculants increased the nodulation and the colonization in most of the treatments. Maximum yield plant^{-1} was recorded in dual and/or triple inoculations under both shade and no shade suggesting that the bio-inoculants used in our study worked synergistically with each other. Thus, the studied bio-inoculants were effective in the open as well as in the shade and can be utilized to overcome the adverse effect of shade to some extent in agroforestry systems.

Keywords Agroforestry systems · Bio-inoculants · Biomass · Intercrops · Mycorrhizal colonization · Nodulation

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Introduction

Agroforestry is an intensive land use management system that integrates trees, shrubs, and crops on a landscape level to achieve optimum benefits (Garrett et al. 1994). Agroforestry lays emphasis not only on beneficial effects of one component on another, but it also involves the manipulations of negative effects to minimize their influence on the productivity of the overall system. At the tree-crop interface of an agroforestry system, trees and crops compete inevitably for light, nutrients, moisture, and other available resources (Shukla et al. 2012a, b).

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Light is the principal limiting factor for the growth of understory vegetation in agroforestry systems, where trees reduce the availability of light to the intercrops (Suresh and Rao 1999; Tomar et al. 2000; Basavraju et al. 2001; Reynolds et al. 2007; Shukla et al. 2009). Bisaria et al. (1996) reported that photosynthetic photon flux density (PPFD) ranged from 1044 to 1215 μmol m^{-2} s^{-1} during rainy season (*kharif*; July to October) and from 701 to 1448 μmol m^{-2} s^{-1} during winter season (*rabi*; November to February) under central Indian conditions. Reported reduction in incident PPFD by the canopies of twelve multipurpose 8-year-old trees (*Acacia nilotica* sp. *indica*, *A. nilotica* sp. *cupressiformis*, *Casuarina equisetifolia*, *Madhuca latifolia*, *Melia azedarach*, *Leucaena leucocephala*, *Dalbergia sissoo*, *Albizia lebbeck*, *Syzygium cumini*, *Eucalyptus tereticornis*, *Emblica officinalis*, and *Hardwickia binata*) ranged from 59.9 to 80.4% during June and from 69.6 to 85.5% during December.

Light intensity has been shown to have a significant influence on rhizobium (RB) and arbuscular mycorrhizal fungi (AMF) (Kumar et al. 2007; Houx et al. 2009; Shukla et al. 2009; Kiran et al. 2013; Sarr et al. 2015). Reductions in number of nodules and dry weight of nodules under low light intensity have been reported (Sarr et al. 2015). Kiran et al. (2013) reported reduced number of nodules and root colonization by AMF in understory crop (*Pisum sativum* L.), growing beneath the canopy of *Dalbergia sissoo* Roxb. Reduction in AMF colonization and growth of important agroforestry trees and crops has also been reported with decrease in light intensity under net-house conditions (Shukla et al. 2009). Tree shade reduces the rate of arbuscular mycorrhizal colonization of intercrops in agroforestry systems under semi arid conditions of central India, especially during winter months (Kumar et al. 2007).

Therefore, to assess the effect of shade on bio-inoculants, viz. RB, phosphate-solubilizing bacteria (PSB), and AMF, which are associated with intercrops in agroforestry systems, an investigation was carried out on important rainy (*Glycine max* (L.) Merr., *Phaseolus mungo* Roxb., and *Vigna radiata* (L.) R. Wilczek) and winter season pulses (*Cicer arietinum* L., *Lens culinaris* Medikus, and *P. sativum*) under net-house conditions with 25% (shade) and 100% (no shade) full sunlight. PSB was included in the study, as it is being recommended to farmers for inoculation of pulses along with RB and AMF under northern and central Indian conditions.

Materials and methods

Site description

The study was conducted at ICAR-Central Agroforestry Research Institute, Jhansi (24° 11′ N and 78° 17′ E), Uttar Pradesh, India. Mean annual rainfall of the region is 960 mm, with an average of 52 rainy days per year. Mean maximum temperature ranges from 23.5 °C (January) to 47.4 °C (June) and mean minimum temperature from 4.1 °C (December) to 27.2 °C (June). May and June are the hottest months. The maximum recorded temperature on a particular day often touches 47–48 °C in the summer. The main soil types at the experimental fields are red and black. Red soil occurs in upland which is shallow, gravelly, and light textured and black soil occurs in comparatively low lying areas which is fine-textured and highly water retentive. Soil pH varies from 5.70 to 6.78 and organic carbon from 0.38 to 0.67%. The topography of the region is undulating. During heavy rains, water stagnates in the low lying areas. Main rainy season crops grown in the area are *P. mungo* (black-gram), *V. radiata* (green-gram), *G. max* (soybean), *Sesamum indicum* (til), *Arachis hypogaea* (groundnut), and *Sorghum bicolor* (sorghum). *Triticum aestivum* (wheat), *Brassica compestris* (mustard), *Hordeum vulgare* (barley), *C. arietinum* (chickpea), *L. culinaris* (lentil), and *P. sativum* (pea) are the main crops grown in the winter season. Pulses are sensitive to water logging; hence, farmers cultivate these in upland plantings during rainy season, and both upland and lowland plantings during winter season.

Biological materials

Seeds of *G. max* (var. JS 93-05) were procured from ICAR-Directorate of Soybean Research, Indore, Madhya Pradesh, India. Seeds of *P. mungo* (var. IPU2-43), *V. radiata* (var. Samrat), *C. arietinum* (var. DCP.92-3), *L. culinaris* (var. DPL-15), and *P. sativum* (var. Vikas) were procured from ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh, India.

Study consisted of three bio-inoculants, viz. RB, PSB, and AMF. Liquid cultures of RB, which were specific to selected crops and liquid culture of PSB (common for all crops) were procured from Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India. Consortium of two AMF species (1:1 ratio, w/w), namely *Acaulospora scrobiculata* Trappe and *Rhizophagus irregularis* (Blaszk., Wubet, Renker and Buscot) Walker and Schubler, served as AMF inoculum, which are common in the region. Their cultures are being maintained in sterilized sand on *Zea mays* L. under net-house conditions. The consortium used in our study consisted of sand along with chopped root bits, spores, and extramatrical mycelium from culture pots.

Application of bio-inoculants

For application of RB (4×10^7 cells ml^{-1}) or PSB (2.2×10^9 cells ml^{-1}), seeds were made sticky with a 1:5 jaggery (a coarse dark brown sugar from sugarcane juice): water (w/v) solution and applied at recommended dose (@50 ml liquid culture for 10 kg seeds). For combined inoculations of RB and PSB, seeds were first coated with RB following the procedure above, then after drying under shade, coated seeds were inoculated with PSB culture. Proper coating of seeds with RB and/or PSB was ensured. Prior to inoculation and sowing, seeds of the test crops were surface sterilized with 0.01% (w/v) $HgCl_2$, washed several (5–6) times with sterilized distilled water, treated with RB and PSB as indicated above and germinated on water agar (8 g l^{-1} w/v) in petri dishes. For placement of the AMF inoculum, a cavity (4–5 cm deep) was made in the soil while filling pots and inoculum was added before closing the cavity and planting germinating seeds on the soil surface.

Net-house experiments

To study the effect of shade on RB, PSB, and AMF, separate experiments were conducted on *G. max*, *P. mungo,* and *V. radiata* during rainy season and *C. arietinum*, *L. culinaris*, and *P. sativum* during winter season in split plot design with seven replications. Treatments included two shade levels (25% (shade) and 100% (no shade) of full sunlight) and eight bio-inoculant treatments (all combinations of RB, PSB, and AMF). The main plots received different levels of light and sub-plots were treated with the bio-inoculants. The sub-plot treatments included all combinations of RB, PSB, and AMF inoculations. To simulate shade conditions which prevail under agroforestry systems, one part of a net-house was shaded by nylon net with 25% light porosity and non-shaded portion of this net-house was used as control. Incident PPFD was reduced by 11.6% in the no shade plot by the iron frame and by 74.1% in the shaded plot by the iron frame and nylon net. Recommended dose of chemical fertilizer (di-ammonium phosphate; DAP, sparingly soluble) was applied in all experimental pots. Experiments with rainy season crops were carried out in red soil (alfisol, sandy loam; EC = 42.7 μS cm^{-1}, Olsen P = 5.6 kg ha^{-1}), whereas experiments with winter crops were carried out in black soil (vertisol, clayey loam; EC = 189.4 μS cm^{-1}, Olsen P = 23.4 kg ha^{-1}). Soil from the experimental farm of the institute was used to fill white plastic pots (24 cm in diameter, 36 cm tall). After germinating seeds were planted, pots were set on a 30 × 30 cm spacing in both the shade and no shade areas of the net-house and watered as needed. Thinning was carried out, leaving one plant per pot after a few days. At maturity, plants were harvested and plant height (cm), dry weight $plant^{-1}$ (g), and yield $plant^{-1}$ (g) were recorded. Dry weight $plant^{-1}$ was recorded after drying the samples in oven at 65 °C, and it included both above ground and below ground biomass (excluding seed yield). Yield $plant^{-1}$ included dry weight of the seeds. The number of nodules $plant^{-1}$ and AMF colonization (Giovannetti and Mosse 1980) were also recorded.

Statistical analysis

Data were subjected to analysis of variance using the ANOVA procedure of the Web Agri Stat Package developed by ICAR Research Complex Goa, India. Statistical significance was determined at the 5% probability level. Means were compared by the least significant difference (LSD) test following a significant *F* test. When an interaction between the two factors was significant, the means of combinations of each level of the factors (simple effects means) were compared.

Results and discussion

Effect of shade on growth and yield

Plant height was higher in full sun than under the corresponding treatment in the shade with few exceptions (Table 1). Dry weight $plant^{-1}$ was lower in all

Table 1 Plant height (cm) of three rainy-season (upper) and three winter-season (lower) pulses inoculated with all eight combinations of rhizobial bacteria (RB), phosphate-solubilizing bacteria (PSB), and arbuscular mycorrhizal fungi (AMF) when grown under 25% (shade) and full (open) sunlight

Inoculum	Light treatment								
	G. max			*P. mungo*			*V. radiata*		
	Open	Shade	Mean	Open	Shade	Mean	Open	Shade	Mean
Non-inoculated	34.3 ± 2.6	40.7 ± 5.3	37.5 ± 5.2	36.3 ± 4.0	41.7 ± 1.1	39.0 ± 4.0	26.4 ± 3.6	24.0 ± 1.2	25.2 ± 2.9
RB	31.6 ± 1.6	44.3 ± 2.0	37.9 ± 6.8	35.0 ± 2.6	62.3 ± 3.3	48.6 ± 14.4	32.8 ± 1.7	34.4 ± 4.5	33.6 ± 3.4
PSB	30.6 ± 1.6	40.9 ± 3.4	35.7 ± 5.9	38.6 ± 3.1	57.1 ± 6.1	47.9 ± 10.6	36.9 ± 2.2	33.3 ± 3.5	35.1 ± 3.4
AMF	29.6 ± 1.8	42.3 ± 1.0	36.0 ± 6.7	43.0 ± 1.5	42.0 ± 1.5	42.5 ± 1.5	37.7 ± 4.1	30.0 ± 3.1	33.9 ± 5.3
RB+PSB	38.1 ± 0.5	39.9 ± 2.1	39.0 ± 1.7	42.5 ± 2.8	60.9 ± 3.5	51.7 ± 10.0	38.3 ± 5.0	30.8 ± 3.4	34.5 ± 5.7
RB+AMF	39.9 ± 1.5	39.1 ± 2.9	39.5 ± 2.3	39.1 ± 3.2	67.3 ± 5.5	53.2 ± 15.2	33.8 ± 2.3	30.4 ± 1.5	32.1 ± 2.6
PSB+AMF	37.1 ± 2.4	35.6 ± 3.4	36.4 ± 2.9	45.3 ± 1.9	63.1 ± 6.4	54.2 ± 10.3	35.4 ± 3.0	33.5 ± 3.1	34.4 ± 3.1
RB+PSB+AMF	36.9 ± 4.1	39.1 ± 3.1	38.0 ± 3.7	44.4 ± 9.6	58.1 ± 1.8	51.3 ± 9.8	36.4 ± 2.3	28.0 ± 3.0	32.2 ± 5.1
Mean	34.8 ± 4.2	40.2 ± 3.8		40.5 ± 5.4	56.6 ± 9.9		34.7 ± 4.7	30.5 ± 4.3	
	C. arietinum			*L. culinaris*			*P. sativum*		
	Open	Shade	Mean	Open	Shade	Mean	Open	Shade	Mean
Non-inoculated	49.9 ± 3.7	29.8 ± 3.2	39.9 ± 11.0	18.6 ± 2.0	27.3 ± 2.8	23.0 ± 5.1	66.9 ± 6.6	73.1 ± 4.3	70.0 ± 6.2
RB	51.7 ± 2.6	42.1 ± 3.6	46.9 ± 4.4	23.7 ± 3.4	28.5 ± 5.7	26.1 ± 5.1	82.4 ± 5.8	110.1 ± 4.9	96.3 ± 15.3
PSB	52.2 ± 4.2	45.6 ± 3.1	48.9 ± 4.9	22.4 ± 2.3	25.2 ± 5.1	23.8 ± 4.1	93.7 ± 4.0	129.6 ± 6.1	111.6 ± 19.3
AMF	52.6 ± 3.6	41.6 ± 3.5	47.1 ± 9.8	24.1 ± 2.9	30.1 ± 2.6	27.1 ± 4.1	87.2 ± 3.3	111.7 ± 6.6	99.5 ± 13.7
RB+PSB	51.1 ± 3.0	44.6 ± 1.3	47.9 ± 4.1	23.8 ± 2.8	24.9 ± 4.2	24.3 ± 3.5	115.4 ± 5.9	92.5 ± 4.3	104.0 ± 12.9
RB+AMF	53.6 ± 4.1	42.8 ± 3.3	48.2 ± 6.6	21.4 ± 2.1	29.4 ± 5.8	25.4 ± 5.9	103.0 ± 8.2	107.7 ± 7.6	105.4 ± 8.0
PSB+AMF	58.9 ± 4.9	42.1 ± 2.9	50.5 ± 9.6	22.8 ± 2.3	25.8 ± 5.3	24.3 ± 4.2	120.9 ± 5.7	108.3 ± 3.6	114.6 ± 8.0
RB+PSB+AMF	46.7 ± 1.0	45.9 ± 4.4	46.3 ± 3.1	21.7 ± 1.8	27.7 ± 6.8	24.7 ± 5.7	112.7 ± 3.0	102.7 ± 8.1	107.7 ± 7.8
Mean	52.1 ± 4.7	41.8 ± 6.3		22.3 ± 2.9	27.4 ± 5.0		97.8 ± 18.3	104.5 ± 16.4	

	$LSD_{0.05}$					
	G. max	*P. mungo*	*V. radiata*	*C. arietinum*	*L. culinaris*	*P. sativum*
Light	1.3	1.8	1.3	1.3	1.0	2.2
Inoculum	2.1	3.1	2.4	2.5	3.1	4.3
Interaction	3.0	4.4	3.4	3.6	4.4	6.1

treatments under shade than the corresponding values in the open for all pulses, except the non-inoculated and PSB in *G. max*. Among treatments in the open, bio-inoculants increased dry weight in all pulses and a more or less similar trend was recorded in the shade (Table 2). The trend of yield plant^{-1} was identical to that of dry weight plant^{-1} (Table 3).

Thus, the results showed that *G. max*, *P. mungo*, *L. culinaris*, and *P. sativum* grown under shade produced taller plants than under open conditions. Our results are consistent with Moniruzzaman et al. (2009), who reported that plants grown in low light levels showed stronger apical dominance than those grown in a high light environment. According to Kurepin et al. (2006), elongation in height could be due to the production of gibberellins in plants that grow under shade. Enhanced plant height under shade represents a cost to resource acquisition through reduction in either root or leaf allocation. Most plants respond to shade by producing leaves that are thinner and usually larger in surface area. It is also an important factor in helping plants to reach enough light (Kumar et al. 2013).

The adverse effects of shade on growth, flowering, and yield of crops have been reported by several other scientists (Cai 2011; Lau et al. 2012; Zhao et al. 2012). Shade decreases photosynthetic capacity of plants, light saturation point, and light compensation point. These decreases cause the soluble sugar, soluble protein, and malondialdehyde contents to decline, which leads to delay in flowering time and thus plant yield. Further, results showed that bio-inoculants increased plant growth and yield in the open as well as in the shade. Beneficial effects of bio-inoculants on growth and yield of plants grown in open sunlight have been reported by several scientists, but information on effect of bio-inoculants on growth and yield under shade is relatively meager. Lau et al. (2012) reported increased biomass in rhizobium inoculated plants grown under shade. Increase in growth of many plant species after inoculation with AMF under different light intensities has been reported by several scientists (Son et al. 1988; Harshi et al. 2004; Shukla et al. 2009).

Maximum yield plant^{-1} was recorded in triple inoculation in all pulses grown in the open whereas in the shade, maximum yield plant^{-1} was recorded in triple inoculation in *G. max*, *V. radiata*, and *P. sativum*, and in double inoculations in remaining crops, PSB+AMF in *P. mungo* and *C. arietinum*, and RB+PSB in *L. culinaris*. In most cases, aforementioned values were superior to yields recorded in single inoculations (Table 3). Thus, the results suggested that bio-inoculants used in our study worked synergistically with each other. Our results are in agreement with existing reports (Khan et al. 1988; Young et al. 1990; Wang et al. 2011; Minaxi et al. 2013; Tagore et al. 2013).

Further, non-statistical comparison of percent increase of dry weight in different treatments over control showed that it was more or less identical under shade and no shade in *G. max*, *P. mungo*, *V. radiata*, *C. arietinum*, and *P. sativum*, and it was more in open in *L. culinaris* (Table 2). In shade, percent increase in yield due to inoculation in different treatments ranged from 35–177% in *G. max*, 12–78% in *P. mungo*, 85–129% in *V. radiata*, 78–146% in *C. arietinum*, 36–140% in *L. culinaris,* and 21–73% in *P. sativum*. In open, the values ranged from 65–201% in *G. max*, 40–97% in *P. mungo*, 52–90% in *V. radiata*, 116–219% in *C. arietinum*, 12–73% in *L. culinaris*, and −2–27% in *P. sativum*. Thus, the efficiencies of bio-inoculants in terms of percent increase in yield over control were comparable to some extent in full sun and under shade in *G. max*, *P. mungo*, and *C. arietinum*, and it was more beneficial in the shade for *V. radiata*, *L. culinaris*, and *P. sativum* (Table 3).

Effect of shade on nodulation and AMF root colonization

In general, rhizobial nodulation was less successful in all treatments under shade than corresponding values in open in all studied pulse crops, except *G. max* and *L. culinaris*. Bio-inoculants increased the nodulations over respective control in treatments under shade as well as in open, except AMF in *G. max* and *C. arietinum* under shade, PSB in *C. arietinum* under shade, and PSB+AMF in *G. max* and *L. culinaris* under shade, and *V. radiata* and *L. culinaris* in open (Table 4). Similarly, AMF colonization was less in treatments under shade as compared to corresponding values in open in all studied crops with a few exceptions. Among treatments in the open, bio-inoculants increased AMF colonization in all pulses, except PSB and RB+PSB in *G. max,* PSB in *P. mungo*, and RB, PSB, and RB+PSB in *C. arietinum*. Among treatments in the shade, all bio-inoculant related treatments increased AMF colonization in *V. radiata*, *C. arietinum*, *L. culinaris*, and *P. sativum* (except RB

Table 2 Biomass (g dry weight plant^{-1}) of three rainy-season (upper) and three winter-season (lower) pulses inoculated with all eight combinations of rhizobial bacteria (RB), phosphate-solubilizing bacteria (PSB), and arbuscular mycorrhizal fungi (AMF) when grown under 25% (shade) and full (open) sunlight

Inoculum	Light treatment								
	G. max			*P. mungo*			*V. radiata*		
	Open	Shade	Mean	Open	Shade	Mean	Open	Shade	Mean
Non-inoculated	9.4 ± 1.0	7.9 ± 0.7	8.7 ± 1.1	35.5 ± 7.2	23.2 ± 1.8	29.4 ± 8.1	35.7 ± 1.9	20.1 ± 2.2	27.9 ± 8.3
RB	17.2 ± 0.9	12.8 ± 0.8	15.0 ± 2.4	51.8 ± 2.0	34.6 ± 3.8	43.2 ± 9.4	49.0 ± 5.7	34.4 ± 3.2	41.7 ± 8.9
PSB	12.7 ± 1.5	12.7 ± 1.5	12.7 ± 1.4	61.1 ± 4.2	42.2 ± 2.0	51.7 ± 10.3	52.6 ± 5.4	29.0 ± 3.5	40.8 ± 13.0
AMF	19.5 ± 2.9	11.0 ± 0.9	15.2 ± 4.9	83.4 ± 3.2	42.2 ± 4.0	62.8 ± 21.7	53.3 ± 4.0	25.4 ± 2.8	39.3 ± 14.8
RB+PSB	20.9 ± 1.6	14.5 ± 0.8	17.7 ± 3.5	78.2 ± 5.7	43.4 ± 2.8	60.8 ± 18.6	47.8 ± 3.3	36.3 ± 3.3	42.0 ± 6.8
RB+AMF	15.1 ± 1.5	12.3 ± 0.8	13.7 ± 1.8	70.1 ± 6.1	39.8 ± 2.8	55.0 ± 16.3	50.3 ± 4.8	28.1 ± 1.5	39.2 ± 12.0
PSB+AMF	20.1 ± 1.3	14.0 ± 1.0	17.0 ± 3.3	77.1 ± 8.2	44.2 ± 1.6	60.6 ± 18.0	60.4 ± 3.1	33.3 ± 2.3	46.8 ± 14.3
RB+PSB+AMF	25.3 ± 1.5	18.8 ± 2.9	22.0 ± 4.0	81.3 ± 2.1	55.9 ± 7.6	68.6 ± 14.2	52.1 ± 4.7	34.9 ± 3.1	43.5 ± 9.7
Mean	17.5 ± 5.0	13.0 ± 3.2		67.3 ± 16.6	40.7 ± 9.4		50.1 ± 7.7	30.2 ± 5.9	
	C. arietinum			*L. culinaris*			*P. sativum*		
	Open	Shade	Mean	Open	Shade	Mean	Open	Shade	Mean
Non-inoculated	19.9 ± 4.0	4.4 ± 0.9	12.2 ± 8.5	8.0 ± 1.0	4.6 ± 1.2	6.3 ± 2.0	20.9 ± 1.2	8.3 ± 1.2	14.6 ± 6.6
RB	30.7 ± 2.7	6.4 ± 0.6	18.5 ± 12.8	16.4 ± 2.7	6.0 ± 1.0	11.2 ± 5.8	30.1 ± 2.9	11.6 ± 1.3	20.8 ± 9.9
PSB	26.1 ± 2.2	7.1 ± 0.7	16.6 ± 10.0	18.2 ± 1.5	6.1 ± 0.6	12.2 ± 6.4	34.4 ± 1.3	12.9 ± 2.6	23.6 ± 11.3
AMF	25.1 ± 2.0	6.1 ± 0.6	15.6 ± 10.0	12.9 ± 1.0	6.3 ± 0.8	9.6 ± 3.5	33.1 ± 3.9	11.9 ± 1.3	22.5 ± 11.3
RB+PSB	28.1 ± 2.8	8.1 ± 0.8	18.1 ± 10.5	20.9 ± 1.9	7.8 ± 1.4	14.4 ± 7.0	35.5 ± 1.7	14.0 ± 2.0	24.8 ± 11.3
RB+AMF	23.4 ± 1.4	6.6 ± 0.5	15.0 ± 8.8	20.7 ± 2.4	6.6 ± 1.3	13.7 ± 7.6	30.9 ± 2.0	11.9 ± 0.8	21.4 ± 10.0
PSB+AMF	27.4 ± 1.8	7.5 ± 0.8	17.4 ± 10.4	23.7 ± 3.4	7.3 ± 1.1	15.5 ± 8.8	33.1 ± 2.9	15.2 ± 1.9	24.2 ± 9.6
RB+PSB+AMF	31.4 ± 2.1	8.2 ± 0.7	19.8 ± 12.1	19.3 ± 1.2	8.7 ± 1.0	14.0 ± 5.6	36.0 ± 0.8	15.2 ± 1.6	25.6 ± 10.9
Mean	26.5 ± 4.2	6.8 ± 1.4		17.5 ± 5.1	6.7 ± 1.5		31.8 ± 5.1	12.6 ± 2.7	

	$LSD_{0.05}$					
	G. max	*P. mungo*	*V. radiata*	*C. arietinum*	*L. culinaris*	*P. sativum*
Light	0.7	3.1	1.5	1.0	0.8	1.1
Inoculum	1.1	3.4	2.8	1.3	1.3	1.5
Interaction	1.6	4.8	3.9	1.9	1.8	2.2

Table 3 Seed yield (g $plant^{-1}$) of three rainy-season (upper) and three winter-season (lower) pulses inoculated with all eight combinations of rhizobial bacteria (RB), phosphate-solubilizing bacteria (PSB), and arbuscular mycorrhizal fungi (AMF) when grown under 25% (shade) and full (open) sunlight

Inoculum	Light treatment								
	G. max			*P. mungo*			*V. radiata*		
	Open	Shade	Mean	Open	Shade	Mean	Open	Shade	Mean
Non-inoculated	2.9 ± 0.2	2.4 ± 0.2	2.6 ± 0.3	13.2 ± 0.5	9.3 ± 0.3	11.2 ± 2.1	9.4 ± 3.6	4.5 ± 0.4	6.9 ± 3.5
RB	7.1 ± 1.0	3.9 ± 0.5	5.5 ± 1.8	18.5 ± 2.8	10.5 ± 1.3	14.5 ± 4.7	14.3 ± 1.8	9.6 ± 1.0	11.9 ± 2.8
PSB	4.7 ± 0.5	5.2 ± 0.8	4.9 ± 0.7	23.2 ± 2.0	16.5 ± 3.2	19.9 ± 4.3	14.9 ± 1.8	8.3 ± 0.9	11.6 ± 3.7
AMF	6.9 ± 0.8	3.2 ± 0.5	5.1 ± 2.0	24.0 ± 2.5	10.7 ± 0.9	17.3 ± 7.1	15.9 ± 1.5	8.6 ± 1.2	12.3 ± 4.0
RB+PSB	6.2 ± 0.4	5.3 ± 0.5	5.7 ± 0.7	25.2 ± 1.5	14.4 ± 0.5	19.8 ± 8.7	16.8 ± 2.3	8.9 ± 0.9	12.8 ± 4.5
RB+AMF	7.1 ± 1.1	5.6 ± 0.9	6.4 ± 1.2	23.1 ± 3.0	13.5 ± 1.2	18.3 ± 5.4	14.5 ± 2.2	8.9 ± 1.1	11.7 ± 3.4
PSB+AMF	6.6 ± 0.9	5.0 ± 0.6	5.8 ± 1.1	24.1 ± 0.6	15.7 ± 2.0	19.9 ± 4.6	15.3 ± 1.7	8.5 ± 0.7	11.9 ± 3.7
RB+PSB+AMF	8.6 ± 0.7	6.7 ± 0.5	7.6 ± 1.2	26.1 ± 2.7	14.1 ± 1.7	20.1 ± 6.6	17.8 ± 3.4	10.3 ± 1.5	14.0 ± 4.6
Mean	6.3 ± 1.8	4.7 ± 1.4		22.2 ± 4.5	13.1 ± 2.9		14.8 ± 3.3	13.1 ± 1.9	
	C. arietinum			*L. culinaris*			*P. sativum*		
	Open	Shade	Mean	Open	Shade	Mean	Open	Shade	Mean
Non-inoculated	3.8 ± 0.6	2.8 ± 0.1	3.3 ± 0.7	5.4 ± 0.5	2.6 ± 0.4	4.0 ± 1.5	12.4 ± 1.3	6.2 ± 0.6	9.3 ± 3.4
RB	10.8 ± 1.8	5.4 ± 0.3	8.1 ± 3.1	6.4 ± 0.8	4.9 ± 0.2	5.6 ± 1.0	13.6 ± 0.9	8.2 ± 0.4	10.9 ± 2.9
PSB	9.2 ± 0.8	6.6 ± 0.3	7.9 ± 1.6	7.1 ± 0.4	3.5 ± 0.4	5.3 ± 1.9	14.6 ± 1.2	7.8 ± 0.6	11.2 ± 3.7
AMF	9.5 ± 0.9	5.0 ± 0.1	7.2 ± 2.4	6.0 ± 0.5	4.1 ± 0.4	5.0 ± 1.1	15.7 ± 3.1	7.5 ± 0.6	11.6 ± 4.8
RB+PSB	11.8 ± 1.3	6.1 ± 0.4	8.9 ± 3.1	8.0 ± 0.7	6.2 ± 0.4	7.1 ± 1.1	14.4 ± 2.5	9.4 ± 0.6	11.9 ± 3.1
RB+AMF	8.3 ± 0.9	6.6 ± 0.7	7.4 ± 1.1	7.5 ± 1.7	4.5 ± 0.4	6.0 ± 2.0	13.5 ± 2.1	8.1 ± 0.4	10.8 ± 3.2
PSB+AMF	9.2 ± 0.7	6.9 ± 0.3	8.0 ± 1.3	9.2 ± 0.7	5.4 ± 0.3	7.3 ± 2.0	12.1 ± 1.5	9.2 ± 0.8	10.6 ± 1.9
RB+PSB+AMF	12.2 ± 1.5	6.4 ± 2.3	9.3 ± 3.2	9.2 ± 0.6	5.7 ± 0.9	7.5 ± 2.0	15.0 ± 2.0	10.7 ± 0.5	12.9 ± 2.7
Mean	9.4 ± 2.7	5.7 ± 1.3		7.3 ± 1.6	4.6 ± 1.2		13.9 ± 2.2	8.4 ± 1.4	

	$LSD_{0.05}$					
	G. max	*P. mungo*	*V. radiata*	*C. arietinum*	*L. culinaris*	*P. sativum*
Light	0.2	0.7	0.5	0.3	0.3	0.9
Inoculum	0.5	1.5	1.5	0.7	0.5	1.0
Interaction	0.8	2.1	2.1	0.9	0.8	1.5

Table 4 Number of rhizobial nodules per plant of three rainy-season (upper) and three winter-season (lower) pulses inoculated with all eight combinations of rhizobial bacteria (RB), phosphate-solubilizing bacteria (PSB), and arbuscular mycorrhizal fungi (AMF) when grown under 25% (shade) and full (open) sunlight

Inoculum	Light treatment								
	G. max			*P. mungo*			*V. radiata*		
	Open	Shade	Mean	Open	Shade	Mean	Open	Shade	Mean
Non-inoculated	6 ± 1	8 ± 2	7 ± 2	85 ± 10	37 ± 4	61 ± 26	31 ± 9	11 ± 1	21 ± 12
RB	11 ± 1	12 ± 1	12 ± 1	270 ± 21	137 ± 17	204 ± 72	62 ± 18	21 ± 4	42 ± 25
PSB	8 ± 1	10 ± 1	9 ± 1	172 ± 20	81 ± 6	127 ± 50	53 ± 15	18 ± 3	35 ± 21
AMF	9 ± 1	8 ± 1	8 ± 1	249 ± 13	70 ± 5	160 ± 93	44 ± 8	15 ± 2	29 ± 16
RB+PSB	16 ± 2	13 ± 2	14 ± 2	229 ± 25	129 ± 26	179 ± 57	73 ± 10	22 ± 3	47 ± 28
RB+AMF	10 ± 1	14 ± 1	12 ± 2	209 ± 24	137 ± 17	173 ± 42	64 ± 20	15 ± 3	40 ± 29
PSB+AMF	12 ± 1	8 ± 1	10 ± 2	172 ± 14	58 ± 10	115 ± 62	34 ± 5	13 ± 2	23 ± 12
RB+PSB+AMF	12 ± 2	13 ± 2	12 ± 2	247 ± 20	122 ± 10	184 ± 67	68 ± 14	21 ± 5	44 ± 26
Mean	10 ± 3	11 ± 3		204 ± 59	96 ± 37		54 ± 20	17 ± 5	
	C. arietinum			*L. culinaris*			*P. sativum*		
	Open	Shade	Mean	Open	Shade	Mean	Open	Shade	Mean
Non-inoculated	26 ± 3	12 ± 2	26 ± 8	19 ± 3	27 ± 6	23 ± 6	82 ± 4	68 ± 6	75 ± 9
RB	65 ± 12	25 ± 7	65 ± 23	40 ± 2	35 ± 7	38 ± 5	262 ± 52	158 ± 13	210 ± 65
PSB	72 ± 13	17 ± 2	72 ± 30	44 ± 3	37 ± 4	41 ± 5	145 ± 19	123 ± 12	134 ± 19
AMF	48 ± 11	19 ± 5	48 ± 17	26 ± 3	35 ± 6	31 ± 7	172 ± 16	121 ± 12	147 ± 30
RB+PSB	109 ± 16	25 ± 2	109 ± 45	65 ± 8	42 ± 8	54 ± 14	177 ± 44	125 ± 10	151 ± 41
RB+AMF	65 ± 17	20 ± 3	65 ± 26	44 ± 6	41 ± 5	42 ± 6	153 ± 21	92 ± 6	123 ± 35
PSB+AMF	77 ± 11	30 ± 7	77 ± 25	21 ± 4	27 ± 5	24 ± 5	127 ± 17	138 ± 7	133 ± 14
RB+PSB+AMF	67 ± 12	27 ± 4	67 ± 26	47 ± 10	43 ± 7	45 ± 9	162 ± 10	148 ± 20	155 ± 17
Mean	66 ± 25	22 ± 7		38 ± 16	36 ± 8		160 ± 55	122 ± 30	

	$LSD_{0.05}$					
	G. max	*P. mungo*	*V. radiata*	*C. arietinum*	*L. culinaris*	*P. sativum*
Light	1	7	4	5	2	8
Inoculum	1	13	7	8	4	16
Interaction	1	18	10	12	6	23

and RB+PSB). In *G. max*, only RB+PSB+AMF and RB+PSB increased the colonization, while in *P. mungo*, the differences in the colonization were non-significant among treatments (Table 5).

Thus, the results suggested that bio-inoculant based treatments increased rhizobial nodulation and AMF colonization in studied crops. In general, maximum nodulation was recorded in RB treated plants (Table 4). Similarly, most of the bio-inoculant based treatments increased AMF colonization. In different crops, maximum colonization was recorded in treatments which involved AMF as one of the inoculants (Table 5). Similar results have been reported by various scientists (Guriqbal et al. 2001; Ballesteros-Almanza et al. 2010).

Non-statistical comparison of percent increase of rhizobial nodulation in different treatments over control showed that it was less in *C. arietinum* and *L. culinaris* in the shade, and more or less comparable in full sun and under shade in the remaining pulses (Table 4). Similarly, the percent increase of AMF colonization was lower in the shade in *G. max*, *P. mungo,* and *V. radiata*, and higher in *C. arietinum* and *L. culinaris*. In *P. sativum*, the values were comparable under both conditions (Table 5). In general, reduction in the nodulation and the colonization was more severe

Table 5 Arbuscular mycorrhizal colonization index (%) of three rainy-season (upper) and three winter-season (lower) pulses inoculated with all eight combinations of rhizobial bacteria (RB), phosphate-solubilizing bacteria (PSB), and arbuscular mycorrhizal fungi (AMF) when grown under 25% (shade) and full (open) sunlight

Inoculum	Light treatment								
	G. max			*P. mungo*			*V. radiata*		
	Open	Shade	Mean	Open	Shade	Mean	Open	Shade	Mean
Non-inoculated	34.7 ± 3.4	33.1 ± 7.8	35.4 ± 4.6	36.4 ± 6.4	41.3 ± 6.1	38.8 ± 7.8	25.5 ± 4.3	31.3 ± 10.4	28.2 ± 9.7
RB	40.5 ± 4.2	31.6 ± 9.5	35.9 ± 0.9	46.0 ± 6.0	39.5 ± 6.3	42.7 ± 7.1	43.6 ± 5.4	40.3 ± 7.4	42.0 ± 7.7
PSB	37.9 ± 1.9	28.9 ± 6.3	33.4 ± 8.1	40.5 ± 6.4	38.1 ± 5.4	39.3 ± 6.1	41.7 ± 8.2	40.3 ± 7.1	41.0 ± 8.1
AMF	50.9 ± 6.6	35.4 ± 6.3	43.1 ± 2.8	55.3 ± 8.7	44.6 ± 3.6	49.8 ± 4.8	49.2 ± 4.8	40.3 ± 5.3	44.6 ± 9.1
RB+PSB	37.9 ± 3.3	37.9 ± 3.7	38.1 ± 7.8	41.6 ± 2.3	38.8 ± 4.1	40.3 ± 5.3	45.0 ± 6.8	38.9 ± 7.4	41.8 ± 8.4
RB+AMF	42.0 ± 5.2	33.7 ± 7.4	37.7 ± 8.0	55.4 ± 8.1	40.8 ± 6.9	48.1 ± 7.4	47.2 ± 11.2	42.5 ± 11.2	44.8 ± 7.6
PSB+AMF	40.8 ± 4.5	35.4 ± 5.8	38.1 ± 7.1	49.2 ± 1.8	36.9 ± 5.7	43.1 ± 2.9	46.9 ± 9.2	42.2 ± 9.5	44.4 ± 11.1
RB+PSB+AMF	40.0 ± 2.9	43.4 ± 6.6	41.7 ± 6.9	59.7 ± 6.2	41.2 ± 6.2	50.6 ± 3.3	54.0 ± 7.7	44.3 ± 10.8	49.2 ± 8.9
Mean	40.7 ± 9.6	34.9 ± 8.7		47.9 ± 8.0	40.1 ± 7.4		43.9 ± 8.7	40.0 ± 8.7	
	C. arietinum			*L. culinaris*			*P. sativum*		
	Open	Shade	Mean	Open	Shade	Mean	Open	Shade	Mean
Non-inoculated	25.5 ± 8.4	16.7 ± 6.1	21.1 ± 8.1	24.4 ± 5.8	15.9 ± 7.6	20.1 ± 8.4	26.4 ± 6.4	23.8 ± 6.5	25.2 ± 6.2
RB	27.5 ± 9.4	23.7 ± 7.8	25.6 ± 7.7	30.8 ± 6.4	25.3 ± 6.6	28.0 ± 6.9	33.1 ± 5.9	24.4 ± 5.2	28.6 ± 8.5
PSB	30.2 ± 3.4	25.8 ± 6.4	27.9 ± 7.4	30.7 ± 6.9	25.9 ± 7.8	28.3 ± 7.9	33.3 ± 9.7	29.1 ± 6.3	31.1 ± 7.6
AMF	33.9 ± 5.6	41.2 ± 6.8	37.4 ± 10.4	31.9 ± 5.9	23.7 ± 8.4	27.8 ± 8.9	35.8 ± 9.4	32.4 ± 7.4	34.1 ± 6.3
RB+PSB	31.0 ± 5.4	30.6 ± 7.1	30.8 ± 8.4	34.4 ± 6.5	31.1 ± 7.4	32.7 ± 9.9	40.8 ± 8.7	28.4 ± 6.9	36.1 ± 8.3
RB+AMF	34.7 ± 6.9	26.1 ± 6.1	30.3 ± 8.6	32.7 ± 5.4	26.6 ± 6.9	29.7 ± 10.4	39.1 ± 7.7	35.2 ± 7.8	37.3 ± 7.4
PSB+AMF	34.4 ± 5.2	30.5 ± 7.8	32.4 ± 7.5	30.3 ± 7.8	26.4 ± 6.4	28.3 ± 8.6	43.2 ± 6.6	39.8 ± 6.5	41.5 ± 6.9
RB+PSB+AMF	36.3 ± 6.2	35.9 ± 6.1	36.1 ± 5.9	43.1 ± 9.4	68.4 ± 5.3	55.9 ± 11.4	47.2 ± 6.8	40.5 ± 7.4	43.8 ± 8.5
Mean	31.6 ± 7.0	28.6 ± 9.4		32.3 ± 8.7	29.9 ± 11.3		37.3 ± 9.6	31.9 ± 9.5	

	$LSD_{0.05}$					
	G. max	*P. mungo*	*V. radiata*	*C. arietinum*	*L. culinaris*	*P. sativum*
Light	4.8	3.3	3.2	2.4	2.2	4.1
Inoculum	3.2	4.1	5.7	6.3	7.4	6.7
Interaction	4.7	5.1	4.9	5.7	3.9	5.2

under shade in winter crops than in rainy season crops. This could be due to low light intensities available during winter as compared to rainy season. Our results are in agreement with the results of Kumar et al. (2007), who reported higher reduction in AMF colonization in understory crops during winter than in rainy season. The results further suggested that correlation between percent increase of dry weight over control (Table 2) and percent increase of rhizobial nodulation over control (Table 4) exist in different crops. Therefore, the inoculation with RB could be critical for biomass production in studied pulses.

In conclusion, shade adversely affected the growth, biomass, and yield of *G. max*, *P. mungo*, *V. radiata*, *C. arietinum*, *L. culinaris*, and *P. sativum*. It also reduced rhizobial nodulations and AMF colonization in aforementioned crops. Application of bio-inoculants increased plant growth for all six pulses whether grown under dense shade or in full sun environment.

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