Journal of Soil Science and Plant Nutrition
https://doi.org/10.1007/s42729-021-00754-z

ORIGINAL PAPER

Efficacy of Arbuscular Mycorrhizal Fungi and Bacterial Inoculants in Enhancing Yield of *Phaseolus mungo* L. and *Vigna radiata* (L.) R. Wilczek under Central Indian Conditions

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Received: 14 July 2021 / Accepted: 23 December 2021

Abstract

The pulse production, stagnating around 22–24 million tonnes in India, is short of present domestic requirement by around 8 million tonnes, and to increase its production, farmers apply excessive dose of chemicals which adversely affect environment and soil as well as human health. To overcome these negative effects, researchers advocate the use of bio-inoculants which may enhance crop yield. Present study, comprising of three bio-inoculants, namely arbuscular mycorrhiza (AM) fungi, rhizobial (Rhi) and phosphate-solubilizing bacteria (PSB), was carried out to test their efficacy in enhancing yield of *Phaseolus mungo* and *Vigna radiata*. In first phase, both crops were inoculated with nine purified fungi. To test the inherent ability of AM fungi, experiment was conducted in autoclaved soil, and to examine competitive ability of the fungi, plants were grown in non-autoclaved soil. Further, in second phase, mixture of better performed AM species (*Acaulospora scrobiculata* and *Rhizophagus irregularis*) was used with bacterial inoculants (Rhi and/or PSB) in pot (net-house) and field (irrigated and rainfed) experiments. Almost all AM species significantly increased crop yield in autoclaved as well as non-autoclaved soils; however, their efficacy was relatively better in autoclaved soil. Integration of AM fungi with Rhi and PSB significantly increased yield in both pot and field experiments. Maximum yield was recorded in the treatment comprising of AM fungi, Rhi and PSB simultaneously. Rhi and AM fungi showed better efficiency under irrigated and rainfed field conditions, respectively. Per cent increase in plot yield in different treatments over control ranged from 20–36% (*P. mungo*) and 26–44% (*V. radiata*) under irrigated and from 23–57% (*P. mungo*) and 20–37% (*V. radiata*) under rainfed field conditions. The results of present study suggested that application of bio-inoculants (AM fungi, Rhi and PSB) can efficiently enhance the yield of both test crops which may bridge the demand and supply gap of pulse crops. However, extrapolation of the results to real field conditions should be done with precaution, because present study was conducted in nutrient-poor soil (Alfisol), and nutrient-rich soil (Vertisol) may affect the outcome of application of bio-inoculants. Therefore, it will be worthwhile to test the efficacy of AM fungi, Rhi and PSB in enhancing the yield of *P. mungo* and *V. radiata* in different soil types.

Keywords Irrigated · Phosphate solubilizing bacteria · Rainfed · Rhizobium · Synergism

1 Introduction

Pulses, a good source of protein (20–25%), provide nutritionally balanced food to vegetarian population (Singh 2017). These can improve physical soil properties with their deep root systems and can also fix atmospheric nitrogen (N) in their root nodules (Stagnari et al. 2017). Pulses can fix approximately 72 to 350 kg N ha^{-1} $year^{-1}$ (Tiwari and Shivhare 2017). In India, these are generally being grown in poor soils with minimum use of resources and water, which make them an integral part of sustainable farming system. In our country, pulses are cultivated in

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Published online: 07 January 2022

both rainy and winter seasons. During 2017–2018, a total of 29.36 million ha area (13.76 and 15.60 million ha during rainy and winter seasons, respectively) was brought under cultivation of pulses; out of which, more than 80% was under rainfed field conditions (Anonymous 2018). This shows that pulses are preferred crop of rainfed agriculture, thus play a significant role in sustaining economy of the rainfed farming community. According to Joshi and Rao (2017), pulses are important crops in the cropping pattern of low-income countries where bulk of the production comes from small farms.

India is the largest producer (25% of global production), consumer (27% of world consumption) and importer (14%) of the pulses in the world (Anonymous 2021). In our country, per capita net availability of the pulses has been declined from 51.1 (1971) to 41.9 g day^{-1} (2013). As per recommendation of the World Health Organization (WHO), minimum requirement of pulses is 80 g $capita^{-1}$ day^{-1}, but the availability of pulses in our country is quite low. To reach self-sufficiency at current population level, India needs to harvest 30 million tonnes of raw pulses whereas our current output is only 22–24 million tonnes (Chandrashekhar 2020).

In our country, various pulse crops, namely *Cicer arietinum* L., *Cajanus cajan* (L.) Millsp., *Vigna radiata* (L.) R. Wilczek, *Phaseolus mungo* L., *Lens culinaris* Medikus, *Pisum sativum* L., *Lathyrus sativus* L., *Vigna unguiculata* L., *Phaseolus vulgaris* L. and *Phaseolus aconitifolius* Jacq., are being cultivated under different agro-ecological conditions (Gowda et al. 2013). Out of these, *P. mungo* (blackgram) and *V. radiata* (greengram), two most important pulse crops of the country, are being cultivated in rainy as well as winter seasons (Singh and Ahlawat 2005). As per an estimate, *P. mungo* was cultivated on 5.44 million ha area and recorded 3.56 Mt production, while *V. radiata* was cultivated on 4.26 million ha area and recorded 2.01 Mt production during 2017–2018 (Anonymous 2018).

In India, cultivation of pulses faces several challenges including farming on marginal lands, dependency on monsoon rain and lack of irrigation facilities, resulting in below normal productivity level. For enhancing the production of pulse crops, farmers use chemical fertilizers along with pesticides and herbicides injudiciously which compound the problem of environmental pollution, soil health deterioration and pesticide residue (Prashar and Shah 2016; Baweja et al. 2020). Adverse effects of these chemicals compelled researchers to look for some alternatives. One such approach could be the use of bio-inoculants, which can save soil, environment and limited resources of the farmers (Bhowmik and Das 2018). The bio-inoculants are low-cost inputs which can stimulate growth and yield of the plants (Swarnalakshmi et al. 2016). Adesemoye and Kloepper (2009) suggested that application of bio-inoculants along with chemical fertilizers can enhance crop productivity.

In Central India, a major pulse growing area of the country, farmers use only inorganic chemical fertilizer, i.e. di-ammonium phosphate (DAP; sparingly soluble) for pulse cultivation which provides 46% P_2O_5 and 18% N. Within short period of time, major portion of the applied phosphorus (P) gets fixed in the soil and becomes unavailable to the plants. So, it is necessary to increase the efficiency of applied P, as it plays an important role in photosynthesis and stimulates symbiotic N-fixation in leguminous crops (Hossain et al. 2017). Availability of the applied P can be increased by applying certain bio-inoculants, e.g. arbuscular mycorrhiza (AM) fungi and phosphate-solubilizing bacteria (PSB). The PSB are known to solubilize sparingly available P compounds into orthophosphate that AM fungi can absorb and transport to the host plant (Shukla et al. 2013a; Nacoon et al. 2020; Rawat et al. 2021). Rhizobium (Rhi), an important bio-inoculant for legumes, helps plant in fixing N through biological N-fixation which ultimately improves growth and yield of the inoculated plants (Getahun et al. 2019). Beneficial effects of these bio-inoculants on crop yields have been reported world over (Toro et al. 1998; Saini et al. 2004; Zaidi et al. 2004; Zaidi and Khan 2006; Meghvansi et al. 2008; Sarawgi et al. 2012; Stancheva et al. 2017; Shukla et al. 2018). In Malwa region of India, Tagore et al. (2013) reported more than 35% increment in yield of *C. arietinum* in treatment consisting of Rhi and PSB, and Singh et al. (2004) reported 49% increment in yield of *V. radiata* when inoculated with AM fungi along with Rhi. Yaseen et al. (2016) also reported approximately 45% increment in yield of *L. culinaris* in treatment having AM fungi and Rhi. However, reports on similar subject in central Indian conditions are quite meager. Hence, present investigation was carried out with hypothesis that the integration of AM fungi with Rhi and PSB will increase the yield of *P. mungo* and *V. radiata* under controlled (net-house) and natural (field) conditions which will not only help in bridging the gap between demand and supply of pulses but also increase income of the small and marginal farmers of central Indian region. For the purpose, we conducted series of experiments under net-house and field conditions. In first phase, response of both crops to AM fungi was assessed in separate pot experiments using autoclaved soil (to evaluate inherent ability of the fungi) and non-autoclaved soil (to evaluate competitive ability of the fungi); and in second phase, better performing AM species was used along with Rhi and PSB, and their effects on crop yield were assessed in pot and field experiments.

2 Materials and Methods

2.1 Site Description

Present study was carried out at Indian Council of Agricultural Research (ICAR)-Central Agroforestry Research

Institute, Jhansi (24° 11′ N and 78° 17′ E), Uttar Pradesh, India, during 2014 to 2016. Mean annual rainfall of the region is 960 mm, with an average of 52 rainy days per year. However, it was recorded only 630, 731 and 657 mm during 2014, 2015 and 2016, respectively. Mean maximum temperature in the region ranges from 23.5 (January) to 47.4 °C (June) and mean minimum temperature from 4.1 (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 (Alfisol) and black (Vertisol). 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 C from 0.38 to 0.67%. The region has two distinct cropping seasons, viz. rainy (July to October) and winter (November to February). In main rainy season, pulse crops grown in the region are *P. mungo*, *V. radiata* and *Glycine max* (L.) Merr., and *Arachis hypogaea* L., *C. arietinum*, *L. culinaris* and *P. sativum* are the main pulse crops grown in the winter season. The topography of the region is undulating, and during heavy rains, water stagnates in the low-lying areas. Pulses are sensitive to water logging; hence, farmers of the region cultivate these in upland plantings during rainy season. Therefore, present study on *P. mungo* and *V. radiata*, which are rainy season crops, was carried out in upland (red) soil.

2.2 Biological Materials

The seeds of resistant varieties of *P. mungo* (var. IPU2-43) and *V. radiata* (var. Samrat) to yellow mosaic disease, a common disease of the region, were procured from ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh, India.

The study consisted of three bio-inoculants, viz. AM fungi, Rhi and PSB. Nine purified AM species, namely *Acaulospora mellea* Spain and Schenck, *Acaulospora scrobiculata* Trappe, *Claroideoglomus etunicatum* (Becker and Gerd.) Walker and Schüßler, *Glomus aggregatum* Schenck and Smith emend. Koske, *Glomus arborense* McGee, *Glomus cerebriforme* McGee, *Rhizophagus fasciculatus* (Thaxt.) Walker and Schüßler, *Rhizophagus irregularis* (Blaszk., Wubet, Renker and Buscot) Walker and Schüßler and *Simiglomus hoi* Berch, were used for inoculation. These species were isolated from the rhizosphere of *P. mungo* and *V. radiata*, growing at research farm of our institute by following the method of Gerdemann and Nicolson (1963). Purified cultures of these species are being maintained in sterilized sand on *Zea mays* L. under net-house conditions at the institute. The cultures of AM species consisted of sand along with chopped root bits, spores and extrametrical mycelium from culture pots. On the other hand, carrier-based liquid cultures of Rhi (*Bradyrhizobium* species 1 and *Bradyrhizobium* species 2 for *P. mungo* and *V. radiata*, respectively) and PSB (*Pseudomonas* spp.; common for both crops), isolated from the fields of *P. mungo* and *V. radiata* in semi-arid, arid and hyper-arid zones of Haryana and Rajasthan state of the India, were procured from the Department of Microbiology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India. These liquid cultures have been produced under ICAR's All India Network Project on Soil Biodiversity-Biofertilizer (AINP SBB), headquartered at ICAR-Indian Institute of Soil Science, Bhopal, Madhya Pradesh, India. The self-life of Rhi and PSB was 1 year and 6 months, respectively.

2.3 Experimental Trials

2.3.1 Screening of AM Fungi in Autoclaved and Non-autoclaved Soils

To test the inherent ability of the AM fungi, an experiment was conducted in autoclaved soil under net-house conditions during rainy season (during July to October) of the year 2014. Study consisted of nine AM species [*A. mellea* (104 spores/50 g sand), *A. scrobiculata* (126 spores/50 g sand), *C. etunicatum* (86 spores/50 g sand), *G. aggregatum* (88 spores/50 g sand), *G. arborense* (92 spores/50 g sand), *G. cerebriforme* (78 spores/50 g sand), *R. fasciculatus* (83 spores/50 g sand), *R. irregularis* (90 spores/50 g sand) and *S. hoi* (77 spores/50 g sand)] and a control. Each treatment was replicated four times in a completely randomized design. Alfisol (sandy loam, pH: 6.29, EC: 134 μS cm^{-1}, organic C: 0.27%, Olsen P: 2.5 ppm) was used as potting substrate. Prior to use, the soil was air dried, sieved through 2-mm sieve to homogenize and separate the roots from soil. Then, soil was autoclaved at 121 °C and 16 psi for 1 h for 2 consecutive days to eliminate naturally occurring AM propagules and other microbes. The autoclaved soil was filled in plastic pots (diameter: 24 cm and height: 36 cm). AM inoculum (50 g) consisting of sand with chopped root bits, spores and extramatrical mycelia was applied 4–5 cm below the seeds, in respective pots. The control pots received an equal amount of autoclaved inoculum. Pre-germinated healthy seedlings of similar sizes were transplanted to each pot. All the pots were transferred to net-house and kept on separate benches. The net-house was fabricated with nylon net allowing 90% sunlight and incident PPFD inside the net-house was 89.4%. The mean maximum temperature during the study period ranged from 31.0 (August) to 32.5 °C (July) and mean minimum temperature from 20.2 (October) to 26.2 °C (July), and the relative humidity varied from 58–82%. Pots were watered as per need and thinning of the plants was carried out, to maintain one plant/pot. At maturity, plants were harvested and observations on plant height (cm), dry weight

(g) plant^{-1}, number of pods plant^{-1} and yield (g) plant^{-1} were recorded. Dry weight plant^{-1} was recorded after drying the samples in oven at 65 °C which included both above- and below-ground biomass (excluding seed yield). Yield plant^{-1} included dry (at room temperature) weight of the seeds. Observations on number of nodules plant^{-1} and root colonization index by AM fungi (Giovannetti and Mosse, 1980) were also recorded. The P content of plants was determined using vanado-molybdo-phosphoric yellow colour method (Jackson 1973) with a UV–VIS spectrophotometer (Perkin Elmer, Lambda 25, Double Beam, USA) at 420 nm and expressed in mg plant^{-1} on the basis of dry weight of the plant. Mycorrhizal dependency (MD; %) was calculated in terms of plant growth as [(M – NM)/M] × 100, using dry weights of individual mycorrhizal plants (M) and mean dry weight of non-mycorrhizal (NM) plants (Plenchette et al. 1983).

To test the competitive ability of abovementioned AM species, similar parallel experiment was carried out in non-autoclaved Alfisol during the same period, i.e. July to October, 2014. Based on the results obtained from these studies, better performing AM fungi species (*A. scrobiculata* and *R. irregularis*) were selected and used for further studies.

2.3.2 Integration of AM Fungi with Rhi and PSB

Pot Study under Net-house Conditions Experiment on integration of AM fungi with Rhi and PSB was carried out under net-house conditions during rainy season (July to October) of the year 2015. A total of eight treatments, viz. AM, Rhi, PSB, AM + Rhi, AM + PSB, Rhi + PSB, AM + Rhi + PSB and control (non-inoculated), were employed. The experiment was conducted in Alfisol, which was filled in plastic pots (diameter: 24 cm and height: 36 cm). Recommended dose of DAP was applied 6–7 cm below seeds in all the pots, and then treatments were imposed in respective pots. Ten times diluted mixture (1:1 ratio, w/w) of *A. scrobiculata* and *R. irregularis*, serving as AM inoculant, was applied 4–5 cm below the seeds. For application of Rhi (4×10^7 cells ml^{-1}) or PSB (2.2×10^9 cells ml^{-1}), seeds were made sticky with the help of 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 inoculation of Rhi and PSB, seeds were first coated with Rhi following the abovementioned procedure, and then after drying under shade, coated seeds were inoculated with PSB culture. All the eight treatments were replicated seven times in completely randomized design, and pots were transferred to net-house and watered as per requirement. At maturity, crops were harvested and observations on plant height (cm), number of pods plant^{-1}, dry weight (g) plant^{-1}, yield (g) plant^{-1}, number of nodules plant^{-1}, root colonization index by AM fungi and shoot P concentration (mg g^{-1}) were recorded. The N determination in plant's shoot was done by Microkjeldahl method (Kjeldahl 1883).

Field Studies Under Irrigated and Rainfed Conditions Field study was conducted at experimental farm of the institute during rainy season (July to October) of the year 2016 in 3 m × 3 m size plots in Alfisol using randomized block design with five replications. A total of six treatments, viz. AM, Rhi, PSB, Rhi + PSB, AM + Rhi + PSB and control (non-inoculated), were included in the study. All the cultural practices recommended for the crops were followed and DAP @ 40 kg P_2O_5 ha^{-1} was applied before sowing in all experimental plots. Crops were irrigated as per requirements. To determine the effect of treatments on growth and yield, ten plants were chosen randomly from central part of each plot at maturity and observations on plant height (cm), above-ground biomass (g) plant^{-1}, number of pods plant^{-1} and yield (g) plant^{-1} were recorded. To exclude the border effect, remaining plants were harvested from 2 m × 2 m quadrat and observation on yield (g) plot^{-1} was also recorded.

Similar study was carried out at farmer's field, approximately 5 km away (village Bhojla of district Jhansi) from the institute, during the same period in 3 m × 3 m size plots in Alfisol using randomized block design with five replications. Crops were cultivated under rainfed conditions. The cultural practices recommended for the crops were followed and observations on above mentioned parameters (field trial under irrigated conditions) were recorded.

2.4 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 of different treatments were compared using Duncan's multiple range test (DMRT). Variation in root colonization index by AM fungi was statistically analyzed for each treatment in different experiments using ANOVA procedure after arcsine transformation of the data.

3 Results

3.1 Screening of AM Fungi in Autoclaved and Non-autoclaved Soils

In autoclaved soil, all AM fungi, except *S. hoi*, increased height of *P. mungo* over control (un-inoculated), and inoculation of *A. scrobiculata* gave significantly higher dry weight and number of pods. Maximum yield was recorded

in *A. scrobiculata* and *R. irregularis* which was comparable with the yield recorded in *A. mellea* and *G. aggregatum*. *A. scrobiculata*-inoculated plant showed significantly higher P uptake. Inoculation of *R. irregularis* showed maximum root colonization index which was found comparable with the index recorded in *G. aggregatum* and *G. arborense*. The MD value ranged from 18.9 to 66.8%, being maximum in *A. scrobiculata* and *R. fasciculatus* (Table 1). More or less, similar results were recorded in *V. radiata*. Higher plant height and dry weight were recorded in *R. irregularis* and number of pods in *G. arborense*-inoculated plant. Inoculation of *R. irregularis* gave maximum yield, P uptake and root colonization index. The MD value ranged from 41.9 to 77.5%, and it was recorded maximum in *R. irregularis* and *G. aggregatum* (Table 1).

In non-autoclaved soil, inoculation of *P. mungo* with *R. irregularis* recorded maximum height and dry weight, and these were statistically comparable with some other AM inoculants. *A. scrobiculata* produced maximum number of pods which was comparable with the pods recorded from inoculation of *G. aggregatum*, *R. irregularis* and *R. fasciculatus*. The maximum seed yield was recorded in *A. scrobiculata* and *R. irregularis*. All AM inoculants significantly increased P uptake over control. Inoculation of *R. irregularis* exhibited significantly higher root colonization index. The MD value for various inoculants varied from 28.5 to 50.1%. Maximum MD was recorded in *R. irregularis* which was comparable with the value recorded in *A. scrobiculata*, *C. etunicatum*, *G. cerebriforme*, *R. fasciculatus* and

Table 1 Effect of arbuscular mycorrhiza fungi inoculation on growth, yield, phosphorus (P) uptake and root colonization index (arc sine transformed) of *Phaseolus mungo* and *Vigna radiata* in autoclaved soil ($n=4$)

Treatments	Plant height (cm)	Dry weight (g) plant^{-1}	Number of pods plant^{-1}	Yield (g) plant^{-1}	P uptake (mg) plant^{-1}	TCI	MD (%)
Phaseolus mungo							
A. mellea	38.8 ± 0.8^{def}	4.76 ± 0.44^{de}	26.5 ± 0.3^{de}	9.62 ± 0.32^{ab}	3.557 ± 0.210^{e}	34.720 ± 0.876^{c}	30.9 ± 5.8^{d}
A. scrobiculata	39.5 ± 0.6^{cde}	9.68 ± 0.23^{a}	33.3 ± 0.9^{a}	9.65 ± 0.15^{a}	10.022 ± 1.057^{a}	37.518 ± 0.678^{bc}	66.8 ± 0.8^{a}
C. etunicatum	38.0 ± 1.1^{ef}	7.30 ± 0.33^{b}	30.0 ± 1.2^{b}	8.77 ± 0.08^{cd}	6.695 ± 0.178^{c}	34.768 ± 0.297^{c}	55.8 ± 2.0^{b}
G. aggregatum	39.9 ± 0.3^{bcde}	4.99 ± 0.27^{d}	29.3 ± 1.3^{bc}	9.41 ± 0.20^{ab}	3.826 ± 0.145^{e}	39.761 ± 0.458^{ab}	35.1 ± 3.3^{d}
G. arborense	43.6 ± 2.0^{ab}	4.08 ± 0.07^{ef}	27.5 ± 0.3^{cd}	9.08 ± 0.10^{bc}	3.513 ± 0.205^{e}	38.700 ± 0.402^{ab}	21.2 ± 1.4^{e}
G. cerebriforme	42.5 ± 0.3^{abcd}	6.09 ± 0.38^{c}	25.5 ± 0.6^{de}	8.42 ± 0.21^{d}	5.231 ± 0.103^{d}	37.756 ± 0.687^{bc}	46.7 ± 3.1^{c}
R. fasciculatus	43.0 ± 1.7^{abc}	7.93 ± 0.51^{b}	24.5 ± 0.5^{ef}	8.31 ± 0.20^{de}	7.252 ± 0.590^{bc}	38.460 ± 0.497^{b}	59.0 ± 2.6^{ab}
R. irregularis	44.0 ± 2.1^{a}	7.41 ± 0.34^{b}	29.5 ± 0.6^{bc}	9.65 ± 0.12^{a}	8.130 ± 0.262^{b}	41.952 ± 0.480^{a}	56.4 ± 1.8^{b}
S. hoi	35.1 ± 2.2^{f}	3.96 ± 0.03^{ef}	22.3 ± 0.9^{f}	7.84 ± 0.16^{e}	3.296 ± 0.132^{e}	36.336 ± 0.404^{bc}	18.9 ± 0.5^{e}
Non-inoculated	35.5 ± 0.5^{f}	3.21 ± 0.14^{f}	11.8 ± 0.9^{g}	3.14 ± 0.21^{f}	1.864 ± 0.295^{f}	5.856 ± 3.388^{d}	
Vigna radiata							
A. mellea	34.5 ± 0.6^{bc}	7.75 ± 0.29^{bc}	24.8 ± 2.0^{d}	12.99 ± 1.04^{bc}	2.573 ± 0.108^{d}	35.681 ± 0.592^{c}	68.8 ± 1.2^{bc}
A. scrobiculata	35.6 ± 0.7^{b}	8.28 ± 0.58^{b}	28.0 ± 1.9^{cd}	15.49 ± 1.55^{ab}	3.247 ± 0.214^{bc}	37.539 ± 0.286^{b}	70.5 ± 2.0^{b}
C. etunicatum	31.5 ± 0.8^{d}	4.30 ± 0.16^{d}	20.0 ± 0.4^{e}	11.06 ± 0.60^{c}	1.869 ± 0.042^{ef}	36.445 ± 0.380^{bc}	43.7 ± 2.1^{e}
G. aggregatum	32.5 ± 0.6^{cd}	10.35 ± 0.43^{a}	29.5 ± 1.7^{bc}	15.49 ± 0.66^{ab}	2.516 ± 0.193^{d}	36.924 ± 0.332^{bc}	76.6 ± 1.0^{a}
G. arborense	29.0 ± 0.4^{e}	4.82 ± 0.29^{d}	34.3 ± 0.9^{a}	13.34 ± 1.49^{bc}	2.099 ± 0.205^{de}	36.369 ± 0.968^{bc}	49.5 ± 2.9^{d}
G. cerebriforme	26.0 ± 0.9^{f}	4.16 ± 0.14^{d}	28.3 ± 0.8^{c}	14.83 ± 0.33^{ab}	2.256 ± 0.280^{de}	37.696 ± 0.896^{b}	41.9 ± 2.1^{e}
R. fasciculatus	33.8 ± 1.3^{bcd}	6.91 ± 0.20^{c}	29.0 ± 1.1^{bc}	15.13 ± 0.48^{ab}	3.518 ± 0.156^{b}	37.073 ± 0.366^{bc}	65.1 ± 1.0^{c}
R. irregularis	39.3 ± 1.2^{a}	10.78 ± 0.52^{a}	30.0 ± 0.8^{bc}	17.17 ± 1.12^{a}	4.844 ± 0.359^{a}	39.554 ± 0.868^{a}	77.5 ± 1.2^{a}
S. hoi	32.4 ± 0.9^{cd}	4.84 ± 0.21^{d}	32.0 ± 0.7^{ab}	16.66 ± 0.51^{a}	2.666 ± 0.094^{cd}	36.100 ± 0.248^{bc}	49.9 ± 2.2^{d}
Non-inoculated	25.4 ± 0.7^{f}	2.41 ± 0.05^{e}	12.5 ± 0.5^{f}	6.56 ± 0.26^{d}	1.487 ± 0.224^{f}	10.674 ± 0.693^{d}	

TCI angular transformed value of colonization index, *MD* mycorrhizal dependency. Different letters in each column represent significant differences between the means among different treatments using one-way ANOVA followed by Duncan's multiple range test ($P<0.05$)

S. hoi (Table 2). In *V. radiata*, maximum plant height was recorded in *R. irregularis*, followed by *S. hoi*, *G. arborense* and *R. fasciculatus*. Maximum dry weight, number of pods and yield were recorded in *A. scrobiculata* which was significantly higher than other inoculants (barring few exceptions). Maximum P uptake was recorded in *C. etunicatum*, followed by *A. scrobiculata*, *G. arborense* and *A. mellea*, and colonization index in *R. irregularis*, followed by *G. aggregatum* and *G. cerebriforme*. The dependency of *V. radiata* on various inoculants varied from 25.5 to 49.7% and recorded maximum in *A. scrobiculata* and *C. etunicatum* (Table 2).

3.2 Integration of AM Fungi with Rhi and PSB

3.2.1 Pot Study under Net-House Conditions

Most of the bio-inoculant-based treatments significantly increased observed parameters in both test crops over control. In *P. mungo*, maximum plant height was recorded in AM + PSB, followed by AM + Rhi + PSB, AM and Rhi + PSB, and dry weight in AM which was comparable with AM + Rhi + PSB and Rhi + PSB. Maximum number of pods was recorded in AM + Rhi + PSB, followed by Rhi + PSB, and yield in AM + Rhi + PSB

Table 2 Effect of arbuscular mycorrhiza fungi inoculation on growth, yield, phosphorus (P) uptake, and root colonization index (arc sine transformed) of *Phaseolus mungo* and *Vigna radiata* in non-autoclaved soil ($n = 4$)

Treatments	Plant height (cm)	Dry weight (g) plant^{-1}	Number of pods plant^{-1}	Yield (g) plant^{-1}	P uptake (mg) plant^{-1}	TCI	MD (%)
Phaseolus mungo							
A. mellea	36.8 ± 0.4^{cde}	8.74 ± 0.26^{cd}	34.8 ± 1.4^{bc}	10.34 ± 0.24^{bc}	4.612 ± 0.489^{b}	38.518 ± 0.499^{d}	39.4 ± 1.8^{bc}
A. scrobiculata	41.9 ± 2.6^{ab}	10.50 ± 0.24^{a}	37.5 ± 0.3^{a}	11.58 ± 0.32^{a}	5.896 ± 0.154^{a}	42.940 ± 0.691^{b}	49.7 ± 1.1^{a}
C. etunicatum	34.0 ± 0.7^{e}	9.82 ± 0.29^{ab}	31.0 ± 0.7^{d}	9.73 ± 0.55^{cde}	6.414 ± 0.329^{a}	40.560 ± 0.394^{cd}	46.1 ± 1.7^{ab}
G. aggregatum	38.1 ± 2.2^{bcd}	7.47 ± 0.44^{e}	35.8 ± 0.5^{ab}	10.03 ± 0.21^{c}	4.385 ± 0.378^{b}	41.978 ± 1.539^{bc}	28.5 ± 4.5^{d}
G. arborense	40.5 ± 1.4^{abc}	7.96 ± 0.47^{de}	33.0 ± 0.9^{cd}	9.96 ± 0.22^{cd}	6.184 ± 0.122^{a}	42.238 ± 0.898^{bc}	32.9 ± 4.0^{cd}
G. cerebriforme	34.9 ± 0.5^{de}	9.79 ± 0.52^{abc}	34.8 ± 0.5^{bc}	9.61 ± 0.17^{cde}	4.848 ± 0.235^{b}	40.833 ± 0.213^{bc}	45.6 ± 3.1^{ab}
R. fasciculatus	34.0 ± 0.7^{e}	9.66 ± 0.45^{abc}	35.3 ± 0.8^{abc}	9.05 ± 0.26^{de}	6.082 ± 0.361^{a}	41.468 ± 0.274^{bc}	45.0 ± 2.7^{ab}
R. irregularis	42.4 ± 1.4^{a}	10.60 ± 0.26^{a}	35.7 ± 0.9^{ab}	11.16 ± 0.38^{ab}	6.765 ± 0.521^{a}	46.100 ± 0.717^{a}	50.1 ± 1.2^{a}
S. hoi	37.4 ± 0.4^{cde}	9.28 ± 0.44^{bc}	32.3 ± 0.9^{d}	8.82 ± 0.45^{e}	4.527 ± 0.120^{b}	38.498 ± 0.906^{d}	42.7 ± 2.8^{ab}
Non-inoculated	36.8 ± 1.5^{cde}	5.28 ± 0.04^{f}	16.5 ± 0.6^{e}	5.78 ± 0.26^{f}	3.308 ± 0.186^{c}	19.683 ± 0.802^{e}	
Vigna radiata							
A. mellea	31.0 ± 0.4^{de}	5.23 ± 0.18^{bc}	21.3 ± 0.9^{c}	11.16 ± 0.50^{c}	6.248 ± 0.357^{ab}	39.080 ± 0.715^{b}	40.1 ± 2.1^{cd}
A. scrobiculata	31.8 ± 0.5^{de}	6.22 ± 0.17^{a}	28.3 ± 0.9^{a}	14.83 ± 0.45^{a}	6.464 ± 0183^{a}	39.670 ± 0.480^{b}	49.7 ± 1.4^{a}
C. etunicatum	32.6 ± 1.2^{cd}	6.10 ± 0.30^{a}	21.0 ± 0.7^{c}	10.53 ± 0.75^{cd}	6.615 ± 0.120^{a}	39.410 ± 0.742^{b}	48.4 ± 2.5^{ab}
G. aggregatum	32.5 ± 1.5^{cd}	4.20 ± 0.10^{e}	17.3 ± 0.9^{d}	9.03 ± 0.39^{de}	5.138 ± 0.268^{cd}	40.360 ± 0.614^{ab}	25.5 ± 1.9^{f}
G. arborense	35.5 ± 1.3^{ab}	4.81 ± 0.20^{cd}	16.3 ± 1.0^{d}	9.10 ± 0.69^{de}	6.435 ± 0.139^{a}	39.780 ± 0.253^{b}	34.8 ± 2.7^{de}
G. cerebriforme	34.5 ± 0.6^{bc}	4.29 ± 0.17^{de}	15.0 ± 1.3^{d}	8.63 ± 0.54^{e}	5.323 ± 0.111^{cd}	40.305 ± 0.681^{ab}	26.9 ± 2.9^{f}
R. fasciculatus	34.6 ± 0.7^{abc}	4.81 ± 0.15^{cd}	22.0 ± 0.7^{bc}	11.55 ± 0.83^{bc}	5.630 ± 0.232^{bc}	38.958 ± 0.181^{b}	34.9 ± 2.0^{de}
R. irregularis	37.3 ± 0.8^{a}	5.44 ± 0.30^{b}	22.5 ± 0.6^{bc}	11.81 ± 0.33^{bc}	4.761 ± 0.465^{d}	42.040 ± 0.764^{a}	42.0 ± 3.6^{bc}
S. hoi	36.8 ± 1.0^{ab}	4.55 ± 0.14^{de}	24.5 ± 1.3^{b}	13.01 ± 0.79^{b}	5.411 ± 0.038^{cd}	38.922 ± 0.241^{b}	31.2 ± 2.1^{ef}
Non-inoculated	29.3 ± 0.5^{e}	3.12 ± 0.08^{f}	11.0 ± 0.4^{e}	7.89 ± 0.35^{e}	3.394 ± 0.060^{e}	20.387 ± 0.940^{c}	

TCI angular transformed value of colonization index, *MD* mycorrhizal dependency. Different letters in each column represent significant differences between the means among different treatments using one-way ANOVA followed by Duncan's multiple range test ($P < 0.05$)

which was comparable with Rhi + PSB, AM and Rhi. Treatments involving rhizobium (Rhi, AM + Rhi and Rhi + PSB) and AM fungi (AM + Rhi + PSB, AM + Rhi and AM) recorded higher number of nodules and root colonization index, respectively. Shoot P and N concentrations were increased by all the treatments and recorded maximum in AM + Rhi + PSB (Table 3). In *V. radiata*, maximum plant height and dry weight were recorded in Rhi + PSB which were comparable with few other treatments. AM + Rhi + PSB produced significantly higher number of pods. Maximum yield was recorded in AM + Rhi + PSB but it was statistically comparable with the yield recorded in AM, Rhi + PSB and Rhi. All treatments, except AM + PSB, significantly increased number of nodules and it was recorded maximum in Rhi + PSB. Two treatments, viz. AM + Rhi + PSB and AM, showed significantly higher colonization index. All bio-inoculant-based treatments significantly increased shoot P and N concentrations and found maximum in AM + Rhi + PSB (Table 3). Results highlighted that treatment consisting of all three bio-inoculants (AM + Rhi + PSB) produced maximum yield in both the crops, while two treatments, viz. AM + Rhi and AM + PSB were not found as efficient as other treatments, viz. AM, Rhi, PSB, Rhi + PSB and AM + Rhi + PSB; hence, these were not taken into consideration for onward studies, under field conditions.

3.2.2 Field Studies Under Irrigated and Rainfed Conditions

Under irrigated field conditions, maximum plant height of *P. mungo* was recorded in Rhi + PSB which was statistically comparable with PSB and Rhi. All treatments significantly increased above-ground biomass; its maximum value was recorded in Rhi, followed by AM. Significantly higher number of pods and yield were recorded in Rhi + PSB. Plot yield was also recorded maximum in Rhi + PSB but it was found comparable with the yield recorded in AM + Rhi + PSB and Rhi. Significantly higher shoot P and N concentrations were recorded in AM + Rhi + PSB and Rhi, respectively (Table 4). In *V. radiata*, plant height and above-ground biomass were recorded significantly higher in Rhi and AM + Rhi + PSB, respectively. Maximum number of pods was recorded in Rhi which was comparable with AM and PSB. Maximum yield plant^{-1} was obtained in Rhi, followed by AM, PSB and AM + Rhi + PSB; all these were comparable with each other. However, plot yield was recorded significantly higher in Rhi and AM. Significantly higher shoot P and N concentrations were recorded in AM + Rhi + PSB (Table 4).

Under rainfed field conditions, bio-inoculant-based treatments significantly enhanced studied parameters over control. In *P. mungo*, significantly higher above-ground biomass was recorded in Rhi + PSB; whereas higher number of pods was observed in Rhi + PSB and AM + Rhi + PSB. Maximum yield plant^{-1} was recorded

Table 3 Effect of application of arbuscular mycorrhiza (AM) fungi with rhizobial (Rhi) and phosphate-solubilizing bacteria (PSB) on growth and yield of *Phaseolus mungo* and *Vigna radiata* under net-house conditions ($n = 7$)

Treatments	Plant height (cm)	Dry weight (g) plant^{-1}	Number of pods plant^{-1}	Yield (g) plant^{-1}	Number of nodules plant^{-1}	TCI	Shoot P concentration (mg g^{-1})	Shoot N concentration (mg g^{-1})
Phaseolus mungo								
AM	43.4 ± 0.6^{ab}	83.34 ± 1.25^{a}	128.9 ± 7.6^{bc}	23.81 ± 0.86^{ab}	71.1 ± 3.2^{d}	43.89 ± 0.69^{a}	44.89 ± 0.16^{abc}	2066.76 ± 31.07^{d}
Rhi	34.6 ± 0.8^{e}	51.39 ± 0.64^{e}	125.9 ± 3.3^{bc}	23.50 ± 0.85^{ab}	139.3 ± 3.4^{a}	40.84 ± 0.32^{bc}	44.54 ± 0.15^{cd}	1808.98 ± 22.58^{e}
PSB	39.1 ± 1.0^{cd}	60.65 ± 1.25^{d}	121.7 ± 3.1^{bc}	23.28 ± 0.67^{b}	80.7 ± 3.3^{c}	38.97 ± 0.43^{de}	44.36 ± 0.1^{de}	1522.35 ± 31.37^{f}
AM + Rhi	39.8 ± 1.0^{bcd}	68.23 ± 1.64^{c}	119.1 ± 3.3^{c}	22.29 ± 0.38^{b}	137.0 ± 4.4^{a}	43.91 ± 0.78^{a}	44.87 ± 0.16^{abc}	2437.98 ± 62.84^{c}
AM + PSB	45.8 ± 0.8^{a}	76.04 ± 2.58^{b}	103.1 ± 2.8^{d}	18.18 ± 0.78^{c}	60.1 ± 3.6^{e}	41.86 ± 0.87^{b}	45.08 ± 0.20^{ab}	2364.93 ± 80.23^{c}
Rhi + PSB	43.1 ± 0.9^{abc}	79.59 ± 2.17^{ab}	133.6 ± 5.7^{ab}	24.01 ± 0.18^{ab}	135.7 ± 2.7^{a}	39.33 ± 0.27^{cd}	44.78 ± 0.14^{bcd}	2873.15 ± 78.29^{b}
AM + Rhi + PSB	45.4 ± 3.2^{a}	82.37 ± 1.16^{a}	143.3 ± 6.1^{a}	25.01 ± 0.47^{a}	125.1 ± 2.7^{b}	45.35 ± 0.38^{a}	45.29 ± 0.19^{a}	3228.85 ± 45.35^{a}
Non-inoculated	35.8 ± 1.2^{de}	34.37 ± 1.93^{f}	79.1 ± 2.6^{e}	13.08 ± 0.19^{d}	37.4 ± 2.6^{f}	37.52 ± 0.43^{e}	44.03 ± 0.04^{e}	796.00 ± 52.28^{g}
Vigna radiata								
AM	35.5 ± 1.4^{ab}	52.46 ± 1.94^{ab}	64.3 ± 2.7^{b}	22.13 ± 0.68^{ab}	46.0 ± 2.6^{c}	44.58 ± 1.09^{a}	45.17 ± 0.14^{ab}	1400.70 ± 51.85^{de}
Rhi	31.1 ± 0.8^{cd}	50.03 ± 1.94^{bcd}	65.6 ± 3.7^{b}	21.46 ± 0.60^{ab}	57.4 ± 4.6^{b}	40.51 ± 0.64^{b}	44.91 ± 0.14^{b}	1481.34 ± 57.46^{cd}
PSB	35.3 ± 1.1^{ab}	50.58 ± 1.89^{bc}	64.6 ± 1.7^{b}	20.65 ± 0.69^{bc}	41.3 ± 2.9^{cd}	40.95 ± 0.69^{b}	44.93 ± 0.15^{b}	1252.50 ± 53.35^{e}
AM + Rhi	31.8 ± 0.9^{bc}	45.12 ± 2.33^{d}	54.7 ± 3.6^{c}	20.28 ± 0.73^{bc}	63.1 ± 1.7^{ab}	41.46 ± 0.20^{b}	45.14 ± 0.04^{b}	1525.35 ± 78.70^{cd}
AM + PSB	35.0 ± 0.8^{ab}	47.23 ± 1.35^{cd}	53.3 ± 2.4^{c}	19.12 ± 0.36^{c}	34.4 ± 1.7^{de}	41.74 ± 0.41^{b}	45.01 ± 0.11^{b}	1750.86 ± 53.29^{b}
Rhi + PSB	37.9 ± 2.0^{a}	56.66 ± 1.73^{a}	67.7 ± 3.0^{b}	23.11 ± 0.98^{a}	69.6 ± 3.0^{a}	40.33 ± 0.85^{b}	45.21 ± 0.11^{ab}	1624.90 ± 46.76^{bc}
AM + Rhi + PSB	33.9 ± 1.2^{bc}	55.19 ± 2.04^{ab}	76.1 ± 2.9^{a}	23.30 ± 0.98^{a}	61.6 ± 4.0^{ab}	45.49 ± 0.80^{a}	45.46 ± 0.07^{a}	2069.01 ± 76.63^{a}
Non-inoculated	27.7 ± 1.8^{d}	38.08 ± 1.16^{e}	34.3 ± 1.4^{d}	14.55 ± 0.83^{d}	27.3 ± 2.0^{e}	39.62 ± 1.03^{b}	44.19 ± 0.05^{c}	826.40 ± 25.15^{f}

TCI angular transformed value of colonization index. Different letters in each column represent significant differences between the means among different treatments using one-way ANOVA followed by Duncan's multiple range test ($P < 0.05$)

Table 4 Effect of application of arbuscular mycorrhiza (AM) fungi with rhizobial (Rhi) and phosphate-solubilizing bacteria (PSB) on various parameters of *Phaseolus mungo* and *Vigna radiata* under irrigated field conditions ($n = 5$)

Treatments	Plant height (cm)	Above-ground biomass (g) plant^{-1}	Number of pods plant^{-1}	Yield (g) plant^{-1}	Yield (g) plot^{-1}	Shoot P concentration (mg) plant^{-1}	Shoot N concentration (mg) plant^{-1}
Phaseolus mungo							
AM	36.3 ± 1.0^{bc}	46.33 ± 1.10^{ab}	73.0 ± 1.6^{bc}	17.80 ± 0.94^{ab}	492.22 ± 6.72^{c}	157.88 ± 0.61^{c}	1288.10 ± 30.61^{c}
Rhi	38.0 ± 0.3^{ab}	48.90 ± 2.08^{a}	70.8 ± 1.5^{c}	17.35 ± 0.49^{ab}	592.05 ± 12.33^{a}	156.04 ± 0.40^{d}	1765.23 ± 75.08^{a}
PSB	38.2 ± 0.6^{ab}	40.18 ± 3.77^{c}	63.8 ± 0.7^{d}	16.48 ± 0.49^{b}	546.53 ± 4.48^{b}	155.02 ± 0.61^{d}	1076.79 ± 100.88^{d}
Rhi + PSB	38.5 ± 0.4^{a}	42.44 ± 1.82^{bc}	83.8 ± 1.4^{a}	19.52 ± 0.64^{a}	609.14 ± 19.14^{a}	159.98 ± 0.47^{b}	1566.02 ± 67.08^{b}
AM + Rhi + PSB	36.0 ± 1.1^{c}	39.42 ± 1.86^{c}	77.8 ± 2.7^{b}	16.43 ± 0.92^{b}	599.80 ± 3.16^{a}	161.68 ± 0.36^{a}	1513.39 ± 71.31^{b}
Non-inoculated	36.0 ± 0.5^{c}	26.19 ± 0.72^{d}	55.6 ± 2.0^{e}	14.11 ± 0.42^{c}	435.76 ± 1.33^{d}	151.18 ± 0.27^{e}	573.52 ± 15.75^{e}
Vigna radiata							
AM	26.6 ± 0.3^{bc}	48.09 ± 0.48^{a}	71.0 ± 0.9^{a}	19.83 ± 0.30^{a}	685.31 ± 3.82^{a}	159.87 ± 0.73^{bc}	1351.20 ± 13.52^{c}
Rhi	27.8 ± 0.2^{a}	44.22 ± 0.73^{b}	71.2 ± 0.4^{a}	20.15 ± 0.13^{a}	688.16 ± 1.51^{a}	158.18 ± 0.74^{cd}	1534.36 ± 25.18^{b}
PSB	26.3 ± 0.4^{bc}	44.26 ± 1.04^{b}	69.2 ± 06^{a}	19.44 ± 0.44^{ab}	625.61 ± 9.25^{b}	157.63 ± 0.42^{d}	1221.45 ± 28.64^{d}
Rhi + PSB	24.8 ± 0.2^{d}	40.85 ± 0.78^{c}	61.8 ± 06^{c}	18.66 ± 0.59^{b}	603.26 ± 4.61^{c}	161.68 ± 0.51^{ab}	1462.33 ± 28.03^{b}
AM + Rhi + PSB	26.7 ± 0.3^{b}	49.98 ± 0.32^{a}	65.2 ± 1.2^{b}	19.39 ± 0.51^{ab}	622.46 ± 2.69^{b}	163.29 ± 0.70^{a}	1864.43 ± 11.81^{a}
Non-inoculated	25.8 ± 0.4^{c}	37.32 ± 1.18^{d}	53.2 ± 1.0^{d}	12.72 ± 0.25^{c}	477.58 ± 2.82^{d}	152.22 ± 0.64^{e}	869.48 ± 27.48^{e}

Different letters in each column represent significant differences between the means among different treatments using one-way ANOVA followed by Duncan's multiple range test ($P < 0.05$)

in AM which was comparable with Rhi + PSB and Rhi. Plot yield was recorded significantly higher in AM + Rhi + PSB. Similarly, shoot P and N concentrations were recorded significantly higher in AM + Rhi + PSB (Table 5). In *V. radiata*, the plant height was recorded maximum in AM + Rhi + PSB which was comparable with Rhi and PSB. The application of AM + Rhi + PSB also resulted in higher above-ground biomass. Maximum number of pods was recorded in AM + Rhi + PSB which was comparable with the values recorded in Rhi + PSB, PSB and Rhi. AM + Rhi + PSB produced maximum yield which was significantly higher than other treatments. Plot yield was also recorded higher in AM + Rhi + PSB but found comparable with AM, Rhi and Rhi + PSB. Significantly higher shoot P and N concentrations were recorded in AM + Rhi + PSB (Table 5).

Table 5 Effect of application of arbuscular mycorrhiza (AM) fungi with rhizobial (Rhi) and phosphate-solubilizing bacteria (PSB) on various parameters of *Phaseolus mungo* and *Vigna radiata* under rainfed field conditions ($n = 5$)

Treatments	Plant height (cm)	Above-ground biomass (g) plant^{-1}	Number of pods plant^{-1}	Yield (g) plant^{-1}	Yield (g) plot^{-1}	Shoot P concentration (mg) plant^{-1}	Shoot N concentration (mg) plant^{-1}
Phaseolus mungo							
AM	37.9 ± 0.5^{a}	42.69 ± 0.65^{b}	72.0 ± 1.6^{b}	18.67 ± 0.24^{a}	359.17 ± 8.02^{b}	156.44 ± 0.36^{c}	1399.28 ± 14.00^{c}
Rhi	39.4 ± 0.8^{a}	41.99 ± 0.55^{b}	62.2 ± 0.9^{d}	18.05 ± 0.22^{ab}	306.79 ± 3.80^{d}	154.57 ± 0.62^{d}	1653.74 ± 27.14^{b}
PSB	38.8 ± 1.0^{a}	42.84 ± 0.27^{b}	68.0 ± 1.6^{c}	17.73 ± 0.14^{b}	325.19 ± 5.62^{c}	154.61 ± 0.29^{d}	1155.06 ± 27.08^{d}
Rhi + PSB	39.5 ± 0.6^{a}	45.73 ± 0.37^{a}	78.2 ± 1.2^{a}	18.33 ± 0.49^{ab}	352.02 ± 10.40^{b}	158.33 ± 0.69^{b}	1588.96 ± 30.46^{b}
AM + Rhi + PSB	38.9 ± 1.0^{a}	41.64 ± 0.23^{b}	78.0 ± 1.0^{a}	16.32 ± 0.34^{c}	393.38 ± 2.79^{a}	160.32 ± 0.25^{a}	1989.39 ± 12.61^{a}
Non-inoculated	40.9 ± 0.7^{a}	31.16 ± 0.41^{c}	65.2 ± 1.2^{cd}	11.99 ± 0.23^{d}	250.04 ± 10.11^{e}	152.15 ± 0.73^{e}	862.02 ± 27.24^{e}
Vigna radiata							
AM	25.2 ± 0.3^{cd}	32.57 ± 0.52^{d}	50.2 ± 2.0^{b}	16.01 ± 0.68^{b}	217.91 ± 10.78^{ab}	158.93 ± 0.78^{bc}	892.28 ± 14.31^{c}
Rhi	26.8 ± 0.3^{ab}	34.62 ± 0.59^{bc}	51.2 ± 1.3^{a}	15.03 ± 0.30^{c}	211.56 ± 6.06^{ab}	157.43 ± 0.66^{cd}	1215.25 ± 20.75^{b}
PSB	26.0 ± 0.9^{abc}	35.37 ± 0.39^{b}	57.0 ± 1.1^{a}	16.30 ± 0.12^{b}	204.71 ± 2.57^{b}	157.28 ± 0.48^{d}	912.55 ± 10.16^{c}
Rhi + PSB	25.8 ± 0.6^{bc}	33.68 ± 0.33^{cd}	57.2 ± 2.2^{a}	16.73 ± 0.10^{b}	211.44 ± 10.73^{ab}	159.33 ± 0.60^{b}	1225.77 ± 12.03^{b}
AM + Rhi + PSB	27.3 ± 0.3^{a}	37.05 ± 0.35^{a}	58.2 ± 0.5^{a}	17.98 ± 0.08^{a}	234.05 ± 4.48^{a}	160.95 ± 0.36^{a}	1393.17 ± 13.27^{a}
Non-inoculated	24.2 ± 0.3^{d}	29.64 ± 0.49^{e}	39.0 ± 0.5^{c}	12.29 ± 0.09^{d}	170.46 ± 7.11^{c}	151.57 ± 0.22^{e}	625.46 ± 10.30^{d}

Different letters in each column represent significant differences between the means among different treatments using one-way ANOVA followed by Duncan's multiple range test ($P < 0.05$)

4 Discussion

4.1 Screening of AM Fungi in Autoclaved and Non-autoclaved Soils

The AM inoculants were efficient in improving growth and yield of *P. mungo* and *V. radiata* in autoclaved as well as non-autoclaved soils. Relatively better growth of AM inoculated plants could be attributed to the increase in soil volume explored for nutrients and water by mycorrhizal plants as compared to non-mycorrhizal ones. According to Diagne et al. (2020), AM inoculants increase the total absorption surface of inoculated plants and thus improve plant access to nutrients, particularly those whose ionic forms have a poor mobility rate or those which are present in low concentration in the soil solution. They also suggested that AM fungi affect plant's morphological and biochemical properties and thus enhance their growth and yield. Better nutrient (especially P) uptake, which is evident from our results, by mycorrhizal plants leads to improved plant biomass and yield (Shukla et al. 2009, 2013b; Jha et al. 2012, 2017). Similar beneficial effects of AM inoculations on growth and yield of *P. mungo* and *V. radiata* have been reported by several researchers under various conditions (Valsalakumar et al. 2007; Ray and Valsalakumar 2010; Mäder et al. 2011; Shukla et al. 2012, 2018). Further, results showed that efficacy of inoculated fungi was comparatively lesser in non-autoclaved soil than that recorded in autoclaved soil. This could be due to increased growth and yield of control plants (un-inoculated) in non-autoclaved soil which might be due to the presence of indigenous AM fungi and other beneficial microbes in non-autoclaved (natural) soil, which were killed by autoclaving in former case. Natural soil generally harbours variety of microorganisms which modulates AM symbiosis and help plants in terms of growth and nutrition management (Meyer and Linderman 1986). In natural soil, mycorrhizal helper bacteria, fungal endobacteria (*Candidatus Moeniiplasma glomeromycotorum*), *Rhizobium radiobacter* and N-fixing endobacteria can modulate the outcome of AM symbiosis (Ray et al. 2020). Relatively lesser efficacy of inoculated fungi in non-autoclaved soil could also be explained on the basis of presence of some deleterious microorganisms in natural soil which might have competed with inoculated AM fungi and suppressed the mycorrhizal activity (Svenningsen et al. 2018; Cruz-Paredes et al. 2019). This is not surprising, as the survival and activity of microbes in any soil system face a monumental task of competing with the myriad of microbes naturally adapted to that same soil (Ray et al. 2020). According to Finkel et al. (2017), introduced microbial inoculant has to compete with indigenous microbes as well as local abiotic conditions.

4.2 Integration of AM Fungi with Rhi and PSB

Various bio-inoculants used under the study efficiently enhanced yield of the test crops. The per cent increase in crop yield in various treatments over control ranged from 39 to 91% (*P. mungo*) and 31 to 60% (*V. radiata*) under net-house conditions, from 20 to 36% (*P. mungo*) and 26 to 44% (*V. radiata*) under irrigated field conditions and from 23 to 57% (*P. mungo*) and 20 to 37% (*V. radiata*) under rainfed field conditions. In all the experiments, simultaneous inoculation of AM, Rhi and PSB produced maximum yield in both the crops, barring few exception. *P. mungo*, grown under irrigated field conditions, exhibited maximum yield in dual inoculated (Rhi + PSB) plot. Higher yield in dual and triple inoculations under different conditions have been reported by various researchers (Erman et al. 2011; Minaxi et al. 2013; Tagore et al. 2013; Ordoñez et al. 2016; Shukla et al. 2018). Sabannavar and Lakshman (2011) reported synergistic effect of AM fungi (*Glomus fasciculatum* and/or *Acaulospora laevis*) when used along with bacterial inoculants (*Azotobacter chroococcum* and/or *Pseudomonas fluorescens*). According to them, bacterial inoculants take part in beneficial effects of AM fungi and improve crop production. Tomar et al. (2001) also reported synergistic interactions between AM fungi, Rhizobium and PSB, and found maximum yield of *Vigna mungo* under field conditions. Our study also suggested that used bio-inoculants worked synergistically with each other under net-house as well as field conditions. According to Artursson et al. (2006), the synergistic interaction between bio-inoculants stimulates growth and yield of plants through various mechanisms which include improved nutrient acquisition (N and P bioavailability) and inhibition of fungal plant pathogens. Higher yield in AM + Rhi + PSB treatment could also be attributed to the production of phytohormones, such as auxins, gibberellins, cytokinins or polyamides (Mittal et al. 2008). Increase in accessibility of trace elements such as siderophore can also explain enhanced crop yield (Wani et al. 2007). In combined inoculation (AM + Rhi + PSB and Rhi + PSB), PSB may solubilize phosphates and release Pi ions from the sparingly soluble inorganic P compounds present in nature into a form that AM fungi may acquire and deliver to the plants (Etesami et al. 2021), and such availability of P could be of use for leguminous plants, as legumes require more P because of high ATP requirement for per mole of N_2 fixation (Vance et al. 2003).

Yield of both test crops were comparatively lesser under rainfed field conditions when compared with yield recorded under irrigated field conditions. Our results agree with the results of Bakhsh et al. (2007), who reported around 15% reduction in seed yield of *C. arietinum* under rainfed field conditions. Similar reduction in seed yield of *V. radiata* has also been reported by Bourgault et al. (2010). Kumaraswamy

and Shetty (2016) suggested that moisture (drought) stress can reduce crop productivity up to 70%. In present study, *P. mungo* and *V. radiata* inoculated with different treatments under rainfed field conditions exhibited 37–92 and 160–225% reduction in plot yield when compared with the yield recorded in respective treatments under irrigated field conditions. This decrease could be due to reduced rate of photosynthesis and nutrient uptake under water stress (rainfed) condition. Water stress affects phenology of the crop, leaf area development, flowering and pod setting which ultimately reduces the crop yield (Malik et al. 2006). Water stress also affects biochemical and physiological processes of the plants which further influences the photosynthetic capacity and growth of the plants that eventually leads to a decline in crop yield (Sharma et al. 2019; Parkash et al. 2021).

Present study reported higher nodulation and root colonization by AM fungi in treatments involving Rhi and AM, respectively, as one of the inoculants. Venkateswarlu (2004) reported higher number of nodules in *V. radiata* and *C. cajan* in treatment having rhizobium, and higher root colonization index in plants treated with AM fungi has been reported earlier in various studies conducted in our laboratory (Chakravarty et al. 2017; Jha et al. 2017; Shukla et al. 2018). Results also suggested that application of bio-inoculants enhanced P and N content in both the crops and these were found maximum in AM + Rhi + PSB. Rhizobium and PSB are highly beneficial in enhancing N and P content in plants because of added N-fixation by rhizobium and the solubilization of native P by PSB (Singh et al. 2011), thus making the two essential nutrients available to the plants by their synergistic effect. AM fungi are well known for P mobillization which increases P availability and enhances activity of rhizobium in rhizosphere, resulting better N and P content in plants (Tajini et al. 2012). Diagne et al. (2020) suggested that root colonization by AM fungi can also improve N content in leguminous plants. Meng et al. (2015) reported 46–50% increment in N uptake by soybean plants after inoculating with AM fungi (*Glomus mosseae*) in various root separation pattern systems.

Further, results of present study suggested that AM fungi and Rhi performed differentially under different water regimes (irrigated and rainfed). AM fungi and rhizobium are morphologically and physiologically different; hence, their responses may also differ in different water levels (Hao et al. 2019). In our study, Rhi performed better under irrigated field conditions which could be due to more number of nodules in well-watered conditions. The greater activity of rhizobium in terms of yield under irrigated conditions has been reported in *C. arietinum* by Singh et al. (2011) in semi-arid region of the India. Erman et al. (2011) suggested that inadequate soil moisture (rainfed conditions) affects the activity and reproduction of rhizobium. Inadequate soil moisture can markedly retard nodule growth and N-fixation and can also lead to morphological and physiological alterations in plants (Ramos et al. 2003). Relatively poor performance of rhizobium under rainfed conditions, as reported in present study, could be due to less formation of root nodules in studied crops. Kumar and Pareek (1984), in a pot experiment, studied the effect of different levels of soil moisture on nodulation and found that *C. arietinum* was unable to form nodules at 10% soil moisture in sandy loam soil, and showed increase in number of nodules with increase in moisture levels from 20 to 50%. On the other hand, AM fungi showed better performance under rainfed field conditions. Our findings correspond with the findings of various researchers (Garcia et al. 2008; Begum et al. 2019). We have also reported beneficial effects of AM inoculation on growth and yield of various plants under low moisture conditions (Shukla et al. 2013b). Possible mechanisms for protecting plants by AM fungi under water stressed conditions could be increased root hydraulic conductivity, enhanced water uptake by extraradical hyphae and improved root size and efficiency, leaf area index and plant's biomass (Erman et al. 2011; Gholamhoseini et al. 2013; Zhang et al. 2018).

5 Conclusion

The results showed that application of AM fungi along with Rhi and PSB had efficiently enhanced the growth and yield of *P. mungo* and *V. radiata*. Specifically, Rhi under irrigated and AM fungi under rainfed field conditions showed better efficiency. Bio-inoculants used under the study, also, resulted in higher yield of *P. mungo* and *V. radiata* even under rainfed field conditions, which can ensure sustainability to the resource poor farmers of rainfed areas. Thus, the results advocated that combined inoculation of AM fungi, Rhi and PSB can be used for enhancing the yield of *P. mungo* and *V. radiata* which will help in bridging the demand and supply gap of pulses in our country. However, it is also suggested that extrapolation of the results to real field conditions should be done with precaution, because present study was conducted in nutrient-poor soil (Alfisol), and nutrient-rich soil (Vertisol) may affect the outcome of application of bio-inoculants. Therefore, it will be worthwhile to test the efficacy of AM fungi, Rhi and PSB in enhancing yield of *P. mungo* and *V. radiata* in different soil types.

Acknowledgements The authors sincerely thank anonymous reviewers for useful comments and suggestions. We are also grateful to the Director, ICAR-Central Agroforestry Research Institute, Jhansi, India for facilitating the research program and constant encouragement during the study. Ashok Shukla acknowledges Science and Engineering Research Board, New Delhi, India for financial support (sanction number: SB/FT/LS-366/2012).

Author Contribution Ashok Shukla conducted entire research work and wrote the article; Anil Kumar formulated the research plan and helped in data analysis; Rajendra Prasad edited the article; Naresh Kumar helped in data collection; S.K. Dhyani edited the article; O.P. Chaturvedi helped in data analysis; and Ayyanadar Arunachalam edited the article.

Funding Science and Engineering Research Board, New Delhi, India for financial support (sanction number: SB/FT/LS-366/2012).

Data Availability Data will be available on request.

Code Availability Not applicable.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

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