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Selected microbial consortia developed for chilly reduces application of chemical fertilizers by 50% under field conditions

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ABSTRACT

The goal of this study was to assess the possibility of reducing chemical fertilizer application through introduction of microbial inoculants for the cultivation of chilly. The microbial inoculants used were *Funneliformis mosseae* and *Bacillus sonorensis* as they revealed strong synergistic relationships and significant improvement of growth, yield and nutrition content of chilly under pot culture studies. Microplot experiment was conducted with selected microbial consortia with varying levels of chemical fertilizers in order to reduce the recommended level of chemical fertilizers for chilly cultivation. The results obtained from the microplot experiment suggested that inoculation with microbial consortia increased plant growth, dry weight of shoot, fruit yield and nutrient concentration. The results also brought out that 50% of recommended NPK fertilizer can be reduced through inoculation with microbial consortia with no adverse effect on growth, nutrition and yield of chilly. The large scale field trial conducted at farmer's field validated the results obtained in the microplot experiment. The soil enzyme activities and soil organic carbon were also more in microbial consortia +50% NPK treatment. Thus the results conclusively brought out that the application of chemical fertilizer to chilly crop can be reduced by 50% which in turn will improve soil health and reduce environmental pollution.

1. Introduction

The greatest challenge of 21st century in many developing countries is to produce the basic necessities especially food for human consumption from the limited available land without soil degradation and using the minimal amount of agriculture inputs. Plant-microbe interactions in the rhizosphere is considered to be the major ecological environment which can be modified to improve plant growth. Research on the use of plant growth promoting rhizobacteria (PGPR) to promote plant growth has increased dramatically over the last few years due to potential benefits observed under field conditions. The mechanisms by which these PGPR enhance plant growth are multitudinous which include production of plant growth-regulating substances, phytohormones, suppression of plant pathogens through antibiosis, bacteriocinogenic action, siderophore production, nitrogen fixation, mineralization of organic phosphorus, production of phytoalexins/flavonoids-like compounds and enhancement of mineral uptake (Chauhan et al., 2015; Mukerji et al., 2006).

The arbuscular mycorrhizal fungi (AMF) in soil are in mutual and beneficial symbiosis with most of the terrestrial plants. The AMF are continuously interactive with a wide range of soil microorganisms including non-bacterial soil microorganisms, PGPR, mycorrhiza helper bacteria and deleterious bacteria. Their interactions can have important implications in agriculture (Miransari, 2011). The interactions between rhizotrophic microorganisms can influence their activities, soil conditions and hence plant growth (Zaidi et al., 2003). Positive effects of mycorrhizal fungi and/or PGPR on plant growth and health as biostimulators, biofertilizers and/or bioprotectors in sustainable agriculture and horticulture were described by many authors (Bagyaraj et al., 2015; Barea et al., 2005; Ryan et al., 2009).

Sustainable agriculture is increasingly viewed as a long-term goal that seeks to overcome problems and constraints that confront the economic viability, environmental soundness, and social acceptance of agricultural production systems worldwide. The use of inorganic fertilizers continuously and excessively, without using organic fertilizers as a balancer may result in soil becoming barren and decrease productivity. "Microbial consortia" is a beneficial microbial mixture that has been developed to improve soil quality

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and crop yield while simultaneously dramatically reducing inorganic chemical application.

Chilly (*Capsicum annuum* L.) is one of the major commercial crops of India with large export potential. India is the largest producer of chilly in the world producing about 1.6 million tons every year (FAOSTATS, 2011). However, the production of chilly in India is sustainably low when a large area (930,000 ha) of production is considered (Bharathi et al., 2004). The application of chemical fertilizers and pesticides in India has been increasing every year to attain the maximum production of chilly. The use of chemical fertilizers has increased 170 fold in last 50 years (FAO, 2010). Since, chilly is a major spice with tremendous export potential, the current emphasis for sustainable cultivation is of importance. A few reports are available using PGPR and AMF separately to improve growth and yield of chilly (Claudia Castillo et al., 2009; Faisal et al., 2010; Moumita et al., 2011). Studies on compatibility of AMF and PGPR and their consequential effect on plant growth, nutrition and yield of chilly at field level has not been carried out so far. This information would assist in the development of microbial consortia for sustainable cultivation of chilly. Developed microbial consortia will not only improve the plant growth and yield of chilly but also help in reducing the application of chemical fertilizers to the soil.

In order to develop a microbial consortia for sustainable cultivation of chilly several AMF were screened for symbiotic response and *Funneliformis mosseae* (*Glomus mosseae*) (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler comb. nov. proved to be best (Thilagar and Bagyaraj, 2015). The AMF culture maintained at culture collection of CNBRCD, Bengaluru, India was used in the study. Similarly several PGPR were screened and *Bacillus sonorensis* (GenBank accession number: KF895394.1) proved to be best in improving growth and yield of chilly (unpublished). Interaction between *F. mosseae* and *B. sonorensis* was then studied and results brought out that interaction is synergistic and dual inoculation with these two as a consortia resulted in better growth and yield of chilly compared to inoculation with either one of them (Thilagar et al., 2014). All these studies were carried out under pot culture conditions. The main objective of this study was to reduce the application of chemical fertilizers by application of selected microbial consortia for sustainable cultivation of chilly. Therefore initially a microplot experiment was conducted with the selected microbial consortia plus varying levels of chemical fertilizer. Based on the results of the microplot experiment, a large scale field trial was conducted to validate the results of microplot experiment.

2. Material and methods

2.1. Microplot experiment

The first investigation was carried out during *kharif* (monsoon season; June–September) 2012–2013 to study the effect of microbial consortia (selected from the pot culture studies) at different levels of NPK fertilizers on growth, plant nutrition, biomass and yield of chilly at microplot level in a farmer's field at Tharabanahalli village near Bengaluru, Karnataka, India. The site was situated at 13°11′ N latitude and 77°38′ E longitude with an altitude of 920 meters above sea level. The temperature during this season ranged from 19.5 °C to 29.1 °C and the radiation ranged from 10.23 to 11.11 MJm^{-2}. The experimental area was prepared and brought to a fine tilth and arranged in a randomized block design, with four replications for the following five different treatments:

T_1: Uninoculated;
T_2: Microbial consortia (MC);
T_3: MC + 50% NPK;
T_4: MC + 75% NPK;
T_5: 100% NPK.

The soil sample from a depth of 0–20 cm was collected from the experimental site before imposing treatments and the soil was analyzed for various physico-chemical properties by adopting appropriate methods. The field soil was red sandy loam with a 6.8 pH, 0.45% organic carbon, 259.94 kg/ha available nitrogen, 28.4 kg/ha phosphorus and 211.6 kg/ha potassium. The size of each plot was 3 m × 3 m. Well decomposed compost at the rate of 3 kg per plot was applied and mixed thoroughly with soil. The recommended level of fertilizer for chilly cultivation is 150:75:75 kg of $N:P_2O_5:K_2O\ ha^{-1}$ respectively. The fertilizers were applied to the plot as per the treatment in the form of straight fertilizers; nitrogen in form of urea, phosphorus in the form of single super phosphate (SSP), potassium in the form of muriate of potash (MoP). Urea contained 46% nitrogen, SSP contained 16% P_2O_5 and MoP contained 60% of K_2O. The amount of urea, SSP and MoP applied to each plot in case of 100% NPK treatment (T_5) were 289 g, 418 g and 112 g respectively. In case of T_3 and T_4, 50% and 75% of these fertilizers were applied respectively. The most commonly used cultivar Namdhari—NS 1701 chilly seeds obtained from Namdhari seeds Pvt., Ltd., Bengaluru, India were used in the study. Chilly seedlings were raised in seedling trays with coco-peat as the substrate. Thirty days old uniform size seedlings were transplanted to the main field with spacing of 75 cm between rows and 30 cm between plants. Plant irrigation was provided by a drip irrigation system which had the spacing of 60 cm between the drippers. Plants were irrigated 2–3 h every day so as to maintain the soil water content at 70–80%. Drip irrigation was not used whenever there was sufficient rain fall.

2.1.1. Microbial consortia inoculation

The microbial consortium consisting of the AM fungus *F. mosseae* and the PGPR *B. sonorensis* (based on our earlier pot culture studies) was used in this investigation. *B. sonorensis* was grown in 2 two-liter flask containing 1.5 L of Luria–Bertani (LB) broth and placed on a rotary shaker for 24 h. The broth after incubation harbored 2×10^8 cfu/mL. *F. mosseae* was multiplied in plastic tubs using soilrite, perlite and vermiculite (1:1:1 v/v/v basis) as the substrate and Rhodes grass (*Chloris gayana*) as the host. The mixture consisting of the root system plus substrate was finely chopped and air dried. The hyphae, spores and root segments present in the air dried substrate served as inoculum. The infective propagules of *F. mosseae* was determined by MPN method (Porter, 1979) and it was found to be 2.8×10^3/g. Microbial consortia comprising *F. mosseae* and *B. sonorensis* were mixed together (which remained as fairly dry inoculum) and applied to the plots as per treatment. Ten grams of microbial consortia was added to each planting hole as per the treatment.

2.1.2. Plant parameters studied

Each plot had 24 plants. Five plants from each plot were randomly selected to study the plant parameters. Plant height and stem girth of the chilly plants were recorded twice [70 and 140 days after transplanting (DAT)]. Biovolume index (BI) (a measure of total volume of a plant) was determined using the formula 'Biovolume index = Plant height (cm) × Stem girth (mm)' given by Hatchell (1985). Chilly fresh fruits were harvested three times (70, 90 and 140 DAT). The plants were uprooted on 140 DAT. For this, shoot of the plants were severed at harvest and kept in an oven at 60 °C for 48 h. Dry weight of shoot was recorded. The concentration of N, P and K in the shoot was determined by methods outlined by Jackson (1973). Roots of the plants were extracted from soil to measure the percent mycorrhizal colonization as described by Philips and Hayman (1970). The AMF spore numbers in the root zone soil were determined by wet sieving and decantation method (Gerdemann and Nicolson, 1963). The soil samples from the root zone were collected from each plot for microbiological and biochemical analyses.