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Growth and yield responses of West Indian lemongrass (*Cymbopogon citratus*) to bio-inoculants under field conditions

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ABSTRACT

The efficacy of commercial microbial treatment on plant growth, nutrient uptake and yield is very well recognized for medicinal plants grown in field conditions. However, the use of commercial and native microbes for *Cymbopogon citratus* has rarely been exploited under field conditions. Therefore, in the present study we evaluated the efficacy of single and native arbuscular mycorrhizal fungal species (AMF) to mixture of mycorrhizal fungal species plus commercial plant growth promoting bacteria (PGPR) on morphological, biochemical and traits of mycorrhizal fungi associated with *C. citratus*. Two native AMF species, i.e. *Glomus mosseae* (G) and *Acaulospora laevis* (A), and *Pseudomonas fluorescens* (P) as commercial PGPR were used in this study. Three different treatments composition of selected microbes, i.e. G, G+P, and G+A+P, were utilized along control for crop production under open field conditions in a randomized complete block design. The plantlets were grown without external application of synthetic fertilizers. The results showed that the mixture of AMF and PGPR significantly increased the survival, biomass, P content of shoot as well as root, essential oil yield, and in vitro antibacterial potential of *C. citratus* against *Escherichia coli* and *Staphylococcus aureus*. In general, this study provides useful insight into the mixture AMF and PGPR treatment that can be applied to improve not only the biomass, phosphorus content, antibacterial potential, and yield attributes of *C. citratus* under open field conditions but also to improve AMF diversity in rhizosphere soil.

Keywords: antimicrobial activity, essential oil content, Glomus, lemongrass, phosphorus uptake, yield attributes

INTRODUCTION

The genus *Cymbopogon* belongs to the Poaceae family, is grown as perennial herb, known for their essential oil having pharmacological as well as therapeutic activities, and is widespread in its distribution throughout tropical and subtropical regions of world (Nambair and Mehta, 2012; Ekpenyong et al., 2014). Out of 144 species of *Cymbopogon*, three species, i.e. *C. citratus* (West Indian lemongrass), *C. flexuous* (East Indian lemongrass) and *C. pendulus* (Jammu lemongrass) are commercially grown in India (Avoseh et al., 2015). The high yield of essential oil per tonne of herbage and drought tolerance of *C. citratus* made it the most popular among all species for cultivation purposes. Lemongrass essential oil is a yellow-coloured viscous liquid, volatile and chemically lipophilic mixture of secondary metabolites, mostly consisting of monoterpenes, sequisterpenes and phenylproponoids. Citral is the main constituent of oil and is made up of geranial and neral isomer; it acts as starting material for synthesis of alpha ionone, beta ionone and beta-carotene. The alpha ionone is used in cosmetics, perfumes, and flavours while beta ionone is used for synthesis of vitamin A. Lemongrass oil has promising antimicrobial, anti-inflammatory and analgesic, antioxidant activity, antispasmodic, antipyretic, diuretic and sedative values (Fokom et al., 2019). It is used as spice due to its good taste appeal and not only used as flavouring agent but also utilized in food preservation and food processing, because of antioxidant, antibacterial, antimycotoxin and antibiofilm action (Boukhatem et al., 2014). The oil also exhibits insecticidal, larvicidal, pesticidal and antifeedant properties (Barbosa et al., 2008). The explosive demand of essential oil for industrial uses and therapeutic potential necessitates getting above average production. In order to get more production, the use of chemical fertilizers is a convenient method but with adverse effect over biological and physical component of an ecosystem. Hence, there is an urgency to adopt alternative strategies to achieve desired biomass production with reduction in use of heavy doses of synthetic fertilizer. The use of microbial inoculants is currently most popularized, cheap and eco-friendly way for attainment of desired production, in spite heavy use of chemical fertilizers (Yadav et al., 2015).

Arbuscular mycorrhizal fungi have been observed to form symbiotic association with medicinal and aromatic plants and play a crucial role in improvement of plant growth parameters in natural and controlled ecosystem. The symbiosis has been reported to influence the absorption of nutrients like phosphorus, zinc and copper, increases water absorption, plant resistance against biological and physical stresses, maintains soil nutrient profile, microbial diversity associated with rhizospheric soil of host plant, stimulates production of growth regulators, synthesis of photosynthetic pigments, and improves osmotic adjustment under stressed conditions (Yadav et al., 2015; Zhu et al., 2001). Due to these beneficial impacts, native AM fungi can be successfully employed for improvement in biomass as well as yield attributes of a plant (Sabannavar and Lakshman, 2009). Similarly, phosphate solubilising and gram negative rhizobacteria *Pseudomonas fluorescens* is well known for plant growth promotion, assistance in AMF establishment and combating the growth of root pathogen within the roots of higher plant. The different investigations have been performed to analyze the synergistic and symbiotic effects of consortium of phosphate solublizing microbes and plant growth promoting rhizobacteria on plants growth, nutrient management and yield attributes under control conditions (Kumar et al., 2020; Kumar et al., 2018). Little information is available on application of native AM fungi either alone or in combination with commercial PGPR over roots of *C. citratus* under open field conditions. So, the present experiment was designed to find out the best and efficient microbial treatment among single native AMF, consortium of native AMF plus nonindigenous PGPR for better growth, phosphorus content, AMF species richness and yield of *C. citratus* under open field conditions.

MATERIALS AND METHODS

Study area and experimental design

The field experiment was performed on sandy loam soil at the Botanical Garden (29˚57' N, 76˚48' E) of Kurukshetra University, Kurukshetra, Haryana, India

from June 2019 to September 2019. Soil chemical and biological properties includes pH - 8.3, EC - 0.65 dS/m, organic carbon - 0.43%, total N - 0.040%, P - 0.033%, K - 0.019%, S - 105.50 ppm, Zn - 2.60 ppm, Fe - 13.52 ppm, Mn - 3.24 ppm, Cu – 2.04 ppm, and approximately 26 AMF spores were distributed in 10g soil sample. Precipitation amount during the field experiment was varying from 83 to 208 mm. The experiment was designed in a random complete block design (RCBD) with a total of four treatments including control grown across 4 separate experimental plots. Each plot has 2×2 meter size and separated by a 0.25-meter-wide path, and thoroughly ploughed up to 15 cm deep. Each treatment was replicated ten times.

Mass multiplication of bio-inoculants

The native dominant AM fungal (*Glomus mosseae* and *Acaulospora laevis*) spores were isolated from the rhizospheric soil of naturally grown *C. citratus* by using wet sieving and decanting technique (Gerdemann and Nicolson, 1963) and multiplied with wheat as host by funnel technique (Menge and Timmer, 1982) for three months. The commercially used inoculum of *Pseudomonas fluorescens* (MTCC NO. 103) was obtained from the Institute of Microbial Technology, Chandigarh, India, and multiplied by using nutrient broth medium having beef extract: 3 g, peptone: 5 g, NaCl: 5 g, 1000 mL distilled water), incubate for 48 hours at 32 ˚C to obtain concentration of 1×10^9 colony forming units (cfu) per mL.

Treatment Composition

In previous work, a pot experiment was performed in polyhouse condition and three treatments were identified as most promising for *C. citratus* (Kumar et al., 2020). These treatments were taken for field trial and are as follow:

T1 Without inoculants (Control)
T2 *Glomus mosseae* (G)
T3 *Glomus mosseae* + *Acaulospora laevis* + *Pseudomonas fluorescens* (G+A+P)
T4 *Glomus mosseae* + *Pseudomonas fluorescens* (G + P)

Transplantation

Transplantation of lemongrass saplings was done in June 2019 containing 10 saplings per plot with respect to treatment. Before transplanting, saplings of treatment T2- T4 were supplemented with AMF inoculum at the rate 50 g/plant around the roots of saplings. Transplanting was done in the evening time and plots were watered immediately after transplantation. In case of T3 and T4 treatments, roots were immersed in nutrient broth medium containing 1×10^9 colony m/L^{-1} concentration of *P. florescence* for 10 minutes. Eradication of weeds was done manually and regularly at a regular interval. After 120 days of transplantation, 5 plants per treatments were randomly uprooted carefully with intact root system and used to records morphological traits.

Analysis of morphological parameters

In Morphological parameters, shoot length (cm), root length (cm), and total leaf area (cm^2) root fresh weight (g), shoot fresh weight (g) were measured at the harvest stage. The collected plants were subjected for oven drying at 70 ˚C, used for shoot and root dry weight assessment.

Estimation of mycorrhization status

AM spores isolation and quantification was done by using standard protocol given by Gerdemann and Nicolson (1963) and Gaur and Adholeya (1994) respectively. The identification of AM fungi was done by using identification manual given by earlier investigators (Schenck and Perez, 1990; Mukerji, 1996). The root colonization was assessed by using protocol designed by Phillips and Hayman (1970) and Giovannetti and Mosse (1980). Root colonization was expressed in percentage and determined by using the following formula:

(Number of root segments colonized/the total number of root segments) × 100

Mycorrhizal inoculation effect was calculated by the following equation of Bagyaraj (1992):

[(Dry weight of inoculated plant - Dry weight of uninoculated plant) / Dry weight of inoculated plant] × 100

Analysis of physiochemical parameters

In physiological parameters, fresh leaf sample was utilized for estimating photosynthetic pigments (chlorophyll) using the protocol given by Arnon (1949). Phosphorus content of plant samples was determined by vanado-molybdate-phosphoric yellow colour method of Jackson (1973), while phosphatase activity was determined by the method of Tabatabai and Bremner (1969). Oil extraction was done by the hydro-distillation method and 25 g fresh leaves sample was subjected to hydro-distillation for two hours in a Clevenger apparatus. Anhydrous sodium sulphate was used to remove moisture of the oil then weighed and stored in vial at +4 ˚C prior analysis. The percent yield of essential oil was calculated by formula:

Yield of essential oil (%) = (Amount of essential oil obtained /Amount of raw materials used) × 100

Antimicrobial activity of AM inoculated plants

Two test microorganisms, i.e. *Escherichia coli* (MTCC1652) and *Staphylococcus aureus* (MTCC96), were procured from Microbial Type Culture Collection IMTECH, Chandigarh. The sub culturing of microorganisms was done on nutrient agar medium and incubated aerobically at 37 °C. For screening for antimicrobial activity, essential oil, acetone, petroleum ether and distilled water leave extracts of AM inoculated *Cymbopogon citratus* was used for evaluation of the antimicrobial activity by the agar well diffusion method (Aneja et al., 2009). A 100 µl of microbial suspension (10^6 CFU mLG^1) was spread onto the agar plates and wells of 5 mm were made in the inoculated agar plates after dry. The powdered dried extracts of treated plant were dissolved in 20% dimethylsulphoxide (DMSO), 50 µL of each extract was poured into the wells of the inoculated agar plates and kept in incubator at fixed temperature (37 °C) for one day. Zone of inhibitions surrounding the well were measured in mm. For MIC determination, a twofold serial dilution of each extract was prepared by first reconstituting 100 mg/mL dried extract in 20% DMSO followed by serial dilution in distilled water to achieve a decreasing concentration range of 50 mg/mL to 0.39 mg/mL. A 100 µL of each dilution was introduced into wells in the agar plates already seeded with 100 µL of standardized inoculum (10^6 CFU mL^{-1}) of the test microbial strain. All test plates were incubated aerobically at 37 °C for 24 h and observed for the inhibition zones. The MIC, expressed as the least concentration of the test extract that completely inhibited the growth of the microbe and showed by a clear zone of inhibition (>8 mm), was recorded for each test organism.

Data analysis

The data was statistically analyzed by using analysis of variance (ANOVA) followed by post hoc test performed by SPSS software package SPSS 16.0 (SPSS Inc. Chicago, IL). Duncan's multiple-range test was performed at P≤0.05 on each of the significant variables measured.

RESULTS

Plant growth response

Shoot height was noticed outstanding in case of T3 (65.25±0.588) followed by T4 (59.57±1.664) (Table 1). Similar kind of observations were recorded in case of root length and root biomass, where the longest root (27.93±0.427) with highest fresh weight (4.531±0.130) and dry weight (1.130±0.030) was noticed in T3 proceeded by T4 for root length (25.61±0.184), fresh weight (2.056±0.012) and dry weight (0.924±0.003). Increment in shoot fresh weight was positively correlated with plant length and observed maximum in consortium inoculation of all selected bio-inoculants (35.16±0.919) followed by dual inoculation of *G. mosseae* and *P. fluorescens* (31.68±0.999) while dry weight was observed maximum in T3 (12.57±0.747) followed by *G. mosseae* that form T2 treatment (12.32±0.388).

Mycorrhization status and mycorrhizal inoculation efficiency

From the data presented in Table 1, it is revealed that the plant growth is directly related to AM spore intensity as well as to the level of root infestation with prevailing AMF spores distributed in rhizosphere of the host plant. All mycorrhizal plants have higher percentage

Table 1. Effect of AM fungi and *P. fluorescens* on growth response *C. citratus* grown under field conditions after 120 days

Parameters	T1	T2	T3	T4	L.S.D (P≤0.05)	ANNOVA F(3,8)
Plant height (cm)	46.15±1.601^{d}	55.15±0.596^{c}	65.25±0.588^{a}	59.57±1.664^{b}	2.3134	129.275
Shoot Fresh weight (g)	15.24±0.526^{d}	29.38±1.032^{c}	35.16±0.919^{a}	31.68±0.999^{b}	1.6805	288.018
Shoot Dry weight (g)	03.67±0.086^{c}	12.32±0.388^{a}	12.57±0.747^{a}	09.31±0.176^{b}	0.8143	280.889
Root length (cm)	15.13±0.260^{d}	19.34±0.679^{c}	27.93±0.427^{a}	25.61±0.184^{b}	0.8139	548.404
Root Fresh weight (g)	1.201±0.032^{d}	1.867±0.048^{c}	4.531±0.130^{a}	2.056±0.012^{b}	0.1354	1234.352
Root Dry weight (g)	0.310±0.001^{d}	0.541±0.009^{c}	1.130±0.030^{a}	0.924±0.003^{b}	0.0302	1585.233
AM spore number/10 g of soil	39.15±1.517^{d}	65.62±1.656^{c}	55.31±1.844^{b}	81.34±1.686^{a}	3.1636	334.103
AM root colonization (%)	22.21±0.460^{d}	41.49±0.261^{c}	66.17±1.670^{a}	58.17±1.677^{b}	2.2838	772.925
AM Inoculation effectiveness (%)	-	69.05	70.94	61.11	-	-

G†: *G. mosseae*, A: *A. laevis*, P: *P. fluorescens*, ‡: Each value is a mean of five replicates, ±: Standard deviation, AM: Arbuscular mycorrhizae, Values in rows followed by same letter are not significantly different, P≤0.05, least significant difference test

of AMF root colonization than control plants colonized by naturally available arbuscular mycoflora in soil with least colonization (22.21±0.460). Among different treatments, maximum root colonization was observed in consortium treatment T3 (66.17±1.670) followed by T4 (58.17±1.677) and single inocula species T2 having individual AMF (41.49±0.261). While maximum AM spore count was reported in T4 (81.34±1.686) followed by T2 (65.62±1.656). A total of eleven AMF species were observed in the soil samples of treated plants and these belong to four genera (*Glomus*, *Acaulospora*, *Gigaspora* and *Entrophosphora*) but their occurrence is variable with treatment (Table 2). Maximum spore diversity was recorded in T3 followed by T4. *Glomus mosseae* and *G. clavisporum* were most frequently occurred species among all treatments. The spore diversity is negatively correlated with total spore number in a specific treatment and positively related with root colonization as more competent spores were available to form symbiosis with root of plants. Maximum mycorrhizal inoculation efficiency (MIE) was calculated for T3 treatment (70.94%) followed by T2 treatment (69.05%) after 120 days of inoculation. So, most efficient treatment is consortium of both AM species plus bacteria and variation reported in treatments are corresponds to nutrient acquisition as well as plant growth credited by mycorrhization (Table 1).

Influences of root colonization on biochemical parameters and yield attributes

The improved morphological traits correspond to the rate of change of physiological and biochemical processes. Plants inoculated with AM fungi were observed with improvement in physiological traits that are under consideration. Root colonization with bio-inoculants increased the level of photosynthetic pigment, i.e. chlorophyll a, chlorophyll b, and total chlorophyll. Lemongrass inoculated with consortium of selected bio-inoculants (2.738±0.1086) had higher total chlorophyll content followed by treatment T4>T2>T1 (Table 3). The other parameters like phosphatase activity of root also increased due to microbial inoculation and observed highest in T4 (acidic phosphatase activity 0.119±0.002 and alkaline phosphates activity 0.145±0.0023) while the minimum was recorded in single inocula treatment. Similarly, P content was recorded as maximum in the same treatment (shoot phosphorus-1.123±0.038 and root phosphorus- 0.625±0.0253) and it corresponds to increased enzymatic activity. The yield attributes were significantly improved by application of indigenous AM fungi plus phosphate solubilizing bacteria and directly correlated with increased shoot biomass, mycorrhization status and P content. The essential oil yield in term of percentage was recorded maximum in consortium, i.e. T3

(1.852±0.031) followed by T4>T2>T1 and in comparison to control 17% increment in yield was recorded in consortium (T3) whereas 12% in single inoculation (T2).

Antimicrobial potential of AM inoculated plants

The results of antimicrobial potential of acetone, petroleum ether, aqueous extracts of *C. citratus* leaves and essential oil, and the positive control ciprofloxacin (for bacteria) are presented in Table 4. The antimicrobial activity of AM treated *C. citratus* leaves extract and essential oil on the agar plates varied in different solvents. The positive control produced significant sized inhibition zones against the test bacteria (ciprofloxacin). Maximum zone of inhibition was recorded in testing extract that belong to T3 treatment, the essential oil performs very well against *Staphylococcus aureus* (19.4 mm) and petroleum ether extract against *Escherichia coli* (26.3 mm). The essential oil extracted from all treatments was effective against both bacteria, followed by petroleum ether, acetone, and aqueous extract. However, water extracts and petroleum ether extract of uninoculated *C. citratus* (T1) do not show inhibitory action against pathogenic bacteria strains. Minimum inhibitory concentration for *Staphylococcus aureus* was reported above 3.125 mg/mL and 6.25 mg/mL for *E. coli* in essential oil extracted from all treatments (Tables 4 and 5).

Table 2. Diversity of AM fungal taxa, frequency of occurrence and richness in different mycorrhizal treatment under field conditions

Sr. No.	Isolated AMF species	Frequency of occurrence	T1	T2	T3	T4
1	*Acaulospora bireticulata*	3	+	+	+	-
2	*A. elegans*	2	+	-	-	+
3	*A. lacunosa*	2	-	-	+	+
4	*A. laevis*	2	-	+	+	-
5	*Entrophospora* sp.	2	+	-	+	-
6	*Gigaspora gigantea*	3	+	-	+	+
7	*G. rosea*	1	-	-	+	-
8	*Glomus clavisporum*	4	+	+	+	+
9	*G. fasciculatum*	3	-	+	+	+
10	*G. macrocarpum*	2	-	+	-	+
11	*G. mosseae*	4	+	+	+	+
	AMF species richness		6	6	9	7

Table 3. Effect of AM fungi and *P. fluorescens* on growth response *C. citratus* grown under field conditions after 120 days

Parameters	T1	T2	T3	T4	L.S.D (P≤0.05)	ANNOVA F(3,8)
Chlorophyll a (mg/g FW)	0.012±0.0003^{d}	0.346±0.0009^{c}	1.367±0.0468^{a}	0.648±0.0023^{b}	0.0441	1820.854
Chlorophyll b (mg/g FW)	0.156±0.0015^{d}	0.472±0.0080^{c}	1.371±0.0568^{a}	0.587±0.0127^{b}	0.0554	923.278
Total Chlorophyll (mg/g FW)	0.168±0.0005^{d}	0.818±0.0346^{c}	2.738±0.1086^{a}	1.235±0.0434^{b}	0.1148	959.882
Acidic Phosphatase activity (I/Ug FW)	0.094±0.003^{d}	0.112±0.002^{b}	0.101±0.001^{c}	0.119±0.002^{a}	0.0051	50.514
Alkaline Phosphatase activity (IUg FW)	0.110±0.0009^{d}	0.132±0.0020^{b}	0.126±0.0025^{c}	0.145±0.0023^{a}	0.0038	149.146
Shoot P(%)	0.251±0.007^{d}	0.654±0.017^{c}	0.813±0.019^{b}	1.123±0.038^{a}	0.0441	720.261
Root P (%)	0.024±0.0002d	0.457±0.0201^{c}	0.512±0.0037^{b}	0.625±0.0253^{a}	0.0307	781.339
Yield of Essential oil (%)	0.810±0.002^{d}	1.523±0.006^{c}	1.852±0.031^{a}	1.797±0.008^{b}	0.0312	4852.977

G†: *G. mosseae*, A: *A. laevis*, P: *P. fluorescens*, ‡: Each value is a mean of five replicates, ±: Standard deviation, AM: Arbuscular mycorrhizae, Values in rows followed by same letter are not significantly different, P≤0.05, least significant difference test

Table 4. Antimicrobial activity of *C. citratus* leaves extract and essential oil on pathogenic microorganisms determined by agar well diffusion method

	Diameter of growth of inhibition zone (mm)							
	Staphylococcus aureus (MTCC96)				*Escherichia coli* (MTCC1652)			
C. citratus extract	PE	DW	ACE	EO	PE	DW	ACE	EO
T1	-	-	-	9.31±0.78	-	-	05.53±0.78	7.32±0.78
T2	14.2± 1.26	10.34±0.78	14.6±0.78	12.5±0.78	12.1±1.26	2.50±0.78	22.15±1.26	18.4±1.26
T3	16.30±0.78	12.5±1.26	18.2±1.26	19.4±1.26	26.3±1.26	5.31±0.78	04.51±0.78	25.5±1.26
T4	-	-	-	15.9±0.78	13.3±0.78	-	-	15.6±0.78
Ciprofloxacin	16.56±0.78				19.53±1.26			

PE petroleum ether, DW distilled water, ACE acetone leaves extract and EO essential oil of leaves of *C. citratus*; -: No activity. The data was analyzed by one-way ANOVA followed by Dunnett's t test compared to positive control at 5% significant level

Table 5. Minimum inhibitory concentration of *C. Citratus* leaves extract and essential oil on pathogenic microorganisms determined by modified agar well diffusion method

	Minimum inhibitory concentration (mg/mL)							
	Staphylococcus aureus (MTCC96)				*Escherichia coli* (MTCC1652)			
C. citratus extract	PE	DW	ACE	EO	PE	DW	ACE	EO
T1	-	-	-	nt	-	-	nt	nt
T2	12.5	50	12.5	3.125	6.25	nt	12.5	6.25
T3	12.5	50	12.5	3.125	3.125	nt	nt	6.25
T4	-	-	-	3.125	6.25	-	-	6.25
Ciprofloxacin	6.25				12.5			

nt- not tested, - : no activity

DISCUSSION

The ability of arbuscular mycorrhizal fungi to accelerate growth and yield of plants by improving the rate of water absorption and nutrient uptake makes them important for sustainable cultivation of therapeutic plants. The surfacing of extraradical hyphae from mycorrhizal plant root system is responsible for the increase of absorptive surface area of the root for improved water absorption and uptake of diffusion limited nutrients, especially of phosphorus (Zou et al., 2015). Similarly, Karagiannidis et al. (2012) found that mycorrhizal inoculation of medicinal and aromatic plants significantly increased shoot and root biomass, leaf biomass, and leaf area. Boutaz et al. (2020) also showed an increase in Na, K, P, and Ca uptake in olive plants inoculated with Rhizolive consortium or *Glomus irregular* for nine months. AM fungus also perform stimulatory role for the synthesis of plant growth hormones especially the auxin that induce lateral root formation owing to take up more nutrient and water (Duca et al., 2014). In the present investigation, a positive relationship was reported between the AM fungi and phosphate solubilising bacteria for growth improvement because of assistive role of *P. fluorescens* in establishment of mycorrhizal infection. These must be the reason for a significant increase in morphological parameters of *C. citratus*. Similar trends of improvement in growth parameters like shoot length, root length and their respective biomass, leaf area and leaf number of mycorrhizal plants grown in field conditions was reported by Fokom et al. (2019).

The tiny root colonization in un-inoculated plant as compared to AMF treatments clearly revealed presence of native AM fungi in experimental soil, significant difference in root colonization advocates the competence of inoculums used in this work to form symbiosis and brings changes in traits of plant. Moreover, the degree of mycorrhizal dependence is relying over host specificity and is imperative for its response to immunization as well as the benefits gained from the mutual association (Urcoviche et al., 2014). Inoculation of lemongrass with mixture of native and commercial bio-inoculants resulted highest sporulation and percent root colonization that might be due to synergistic interaction between *P. fluorescens* and AM fungi. Increased mycorrhization directly related to symbiotic intimacy between fungus and inoculated plant and leads to exchange of nutrients. However, the extent of mycorrhization in inoculated plant is majorly constrained by availability of phosphate in the soil. High phosphate concentration in the soil adversely affects bio-fertilizer potential of AM fungi through controlling spore germination and multiplication, carbon supply to the developing hyphae and its growth from germinating spores (Zubek et al., 2012). Moreover, mycorrhizal inoculation effect clearly showed effectiveness of specific treatment and observed maximum in consortium of bio-inoculants. Similar investigations in search of efficient mycorrhizal colonization by AM fungi were performed on *Garcinia indica* and on pepper, tomato, and eggplant (Lakshmipathy et al., 2003; Ortas, 2012).

High concentration of chlorophyll pigments in leaves of AM inoculated plants is associated with an increase in the number and size of chloroplasts which improved carbon assimilation, increased uptake of phosphorus and magnesium, stomatal conductance, and increased transpiration (Arumugam et al., 2011). The increased uptake of phosphorus by AM treated *C. citratus* plants confirms that the plants have a high degree of reliance on symbiosis phenomenon for better uptake of phosphorus for achieving better growth and high yield. Our results confirm the findings of Çekiç et al. (2012), who observed an improvement in phosphorus and chlorophyll content in AM treated plants as compared to control. Babaei et al. (2012) demonstrated a positive relationship between AMF and phosphate solubilising bacteria that brings maximum nutrient acquisition in inoculated plants of *Helianthus annuus*. Likewise, Vafadar et al. (2014) reported improved total chlorophyll content in *Stevia robusta* when inoculated with combination of AM fungi and N-fixing bacteria. Our results confirm the findings of Rouphael et al. (2015), who also observed an improvement in synthesis of hydrolases and phosphatases enzymes in inoculated plants in comparison to control, and this improvement might be a reason of improved phosphatase activity as well as P uptake. The acidic phosphatase activity

determines the mobilization and uptake of phosphorus from rhizosphere, and alkaline phosphatase activity regulates active transport of phosphate to mycorrhizal roots (Tanwar et al., 2014). This increased phosphatase activity contributes to increased shoot and root P in the host plant grown in nutrient deficient soil. Inorganic phosphorus is less mobile in nature but application of mycorrhizal fungi successful made it susceptible for mobilization and allowing co-transport of phosphorus to host plant.

In general, the climatic factors in which the plants grown had great influence on the biochemical process, causing changes in existing level of the active compounds and promoting biosynthesis of new compounds that may finally alter the essential oil content. The mycorrhizal plants showed variation in quantity and quality of essential oil that might be due to change in biological and biochemical parameters. The synthesis of essential oil is linked with P nutrition, and it gets improved in plants inoculated with AM fungi (Ashour et al., 2010). A similar increment in quantity of essential oil in various plant species following AMF infestation as compared to non-inoculated plants was observed by different workers (Karagiannidis et al., 2012; Tarraf et al., 2015). Oliveira et al. (2019) observed slight increase in sesquiterpene hydrocarbon production (myristicin 18.38–19.93% and elemicin 2.22–3.13%) in AM inoculated *Piper aduncum*. The results of various studies stipulated that an increased level of inorganic phosphorus is responsible for increment in secondary metabolites (Singh et al., 2009). Maximum oil yield in consortium of AMF and PGPR inoculated *C. citratus* was in accordance with Horii and Ishii (2014) who investigated the combined effect of *G. clarum* and *Pseudomonas* sp. on the secondary metabolites of sesame plants and observed maximum oil yield and sesamin content in the seeds than single inoculation. We observed that the *C. citratus* essential oil extracted from treatment T3 displayed the maximum antimicrobial activity against the microorganisms tested. This may be due to altered chemical composition of oil by bio-inoculants, solubility of the active compounds in organic solvents and their diffusion rates which influences the zone of inhibition observed in study (Fokom et al., 2019). Similar investigation was done by Morelli et al. (2017) who observed the influence of AM fungi and humic substance over oil yield of *Ocimum basilicum* (L.) and its antibacterial activity against some pathogenic bacteria and recorded lowest MIC against *Bacillus cereus*. The experimental findings are acting as a foundation for future research concerned with identification of causative bioactive compound for claimed antimicrobial potential.

CONCLUSION

In this experiment, inoculation with bio-fertilizers of field-grown lemongrass (*Cymbopogon citratus*) and conglomerate treatment of native AMF and commercial PGPR gave the best results in overall growth of plants. These results confirm the synergistic and symbiotic efficiency of bio-inoculants for experimental plants, responsible for the promotion of root and shoot biomass, incrementing in photosynthetic pigments, nutrition content, improving the essential oil content, and affect antimicrobial activity with consequences on rhizospheric microbial diversity. The role of bio-fertilizers is a key issue to be considered during the production of this plant in a cost effective as well as sustainable way.

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