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Efficacy of bioinoculants on biomass, nutritional status and yield of lemon grass, *Cymbopogon citratus* (DC.) Stapf.

A Kumar[1,*], A Gupta[2], A Aggarwal[1] & V Bhargav[1]

[1]*Department of Botany, Kurukshetra University, Kurukshetra-136 119, Haryana.*
[2]*Institute of Integrated and Honors Studies, Kurukshetra University, Kurukshetra-136 119, Haryana.*
**E-mail: ashishbotany990@gmail.com*

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Abstract

In the present investigation, different bioinoculants were analysed for their efficacy as bio-fertilizer for lemongrass (*Cymbopogon citratus* (DC.) Stapf.) under controlled conditions. Bioinoculants including dominant arbuscular mycorrhizal fungi (*Glomus mosseae* and *Acaulospora laevis)* and phosphate solubilizing bacteria (*Pseudomonas fluorescens)* were used in the experiment. The effects of these bioinoculants applied alone or in combinations were assessed on one month old saplings of *C. citratus.* The results showed that application of bio-inoculants significantly influenced growth, biomass production, biochemical properties and yield parameters. A consortium of *G. mosseae*, *A. laevis* and *P. fluorescens* showed best results for plant height, root length and biomass. Mycorrhization status was highest in the consortium treatment. The biochemical and yield (biomass) parameters were also found to be better with the application of consortium.

Keywords: *Glomus mosseae*, *Acaulospora laevis*, *Cymbopogon citratus*, phosphatase activities, essential oil

Introduction

Lemon grass (*Cymbopogon citratus* (DC.) Stapf.) belonging to the family Poaceae is one of the most commonly grown perennial herb for its aromatic and therapeutic values throughout tropical and subtropical regions of world (Nambiar & Matela 2012). The plant is a native herb of India, flourish well in loamy soil with optimum dose of fertilizer i.e. 60:45:35 kg ha^{-1} N, P_2O_5 and K_2O basally and 60 kg N in 3 to 4 splits $annum^{-1}$ as top dressing and day temperature of 25 to 30°C temperature. Leaves of the plant have been commercially exploited for their essential oil and are enriched with citral as major constituent. Citral, a combination of geranial and neral isomers is used as a raw material for the production of vitamin A, ionone, beta-carotene

and has strong antimicrobial properties besides pheromonal effects in insects (Robacker & Hendry 1977). The oil extracted from *C. citratus* used in cosmetic products, herbal tea made from leaves have some medicinal values like anti-inflammatory and analgesic (Ortiz *et al*. 2002), antispasmodic, antipyretic, diuretic and sedative. Lemon grass has been used as spice and flavouring agent in food due to its taste appeal, and as folk medicine in several regions of the world for treatment of inflammation, nervous disorder, gastrointestinal disturbances, fever and hypertension (Nambiar & Matela 2012). Although its value is significant its productivity is low. Hence, quantitative improvement in essential oil production will help to compensate ever increasing demand for essential oil from commercial point of view. Use of bio-fertilizer is currently most popularized, which is a cheap and environment friendly approach for successful accomplishment of improvement in oil production as well as biomass, despite the heavy use of inorganic fertilizers (Yadav *et al*. 2015). Phosphate solubilizing bacteria *Pseudomonas fluorescens* and Arbuscular Mycorrhizal fungi (AMF) are known as effective biofertilizers in this process (Sabannavar & Lakshman 2009). Different investigations have been performed to find out the additive effects of both phosphate solubilizing microorganisms and plant growth promoting rhizobacteria on plant growth as well as yield of medicinal plants (Yadav *et al*. 2015; Lermen *et al.* 2019). Many other aspects of arbuscular mycorrhizal interactions including biocontrol towards plant pathogen, tolerance to water stress and adverse environmental conditions were studied, but little is known about their potential effect on the quantitative and qualitative profile of the secondary metabolites (e.g. essential oils) in medicinal and aromatic plants (Morone-Fortunato & Avato 2008).

Earlier works studied the effects of arbuscular mycorrhizal fungi (*Rhizophagus clarus* and *Claroideoglomus etunicatum*) inoculation on growth performances under contrasting phosphorus level (Lermen *et al.* 2019). Fokom *et al.* (2019), studied the effect of harvesting period and AM fungi on growth, essential oil content and antioxidant properties of lemon grass grown under field conditions. Lermen *et al.* (2015), studied the effect of bio-fertilizer on content as well as chemical composition of essential oil of *C. citratus* grown under different levels of lead and Burni *et al*. (2013), studied the effect of arbuscular mycorrhizal fungi on essential oils in lemongrass grown in marginal soils.

The present investigation was concerned to examine the influence of phosphate solubilizing bacteria such as *P. fluorescens* on colonization of *C. citratus* roots by different AM fungi and secondly to see the effect of bio-fertilizer alone or in combination in uptake of P and its influence on growth and yield of *C. citratus*. The application of *Glomus mosseae* and *Acaulospora laevis* either alone or in combination with *P. fluorescens* and lack of external application of synthetic fertilizer to the soil made it different from earlier studies. The objective of this study was to assess the efficacy of AM inoculum and phosphate solubilizing bacteria alone or in combination on growth, physiology and essential oil yield of *C. citratus*.

Material and methods

The study was conducted in a randomized block design with eight treatments replicated five times, during December 2016 to March 2017 in a polyhouse at the Botany Department, Kurukshetra University, Kurukshetra, Haryana, India. The polyhouse received natural sunlight with controlled temperature (25°±5°C) and humidity (50-70%). The soil characteristics were as follows: loamy soil type having sand -64.2%, clay -3.90%, silt -21.81%, pH -8.08±0, EC -0.25 dS m^{-1}, organic carbon -0.40%, nitrogen -0.042%, phosphorus -0.017% and potassium -220 kg ha^{-1}.

The dominant AM fungal spores (*G. mosseae* and *A. laevis*) were isolated from the rhizospheric

soil of field grown *C. citratus* plants by using Wet Sieving and Decanting Technique of Gerdmann & Nicolson (1963) and identified by using keys of Schenck & Perez (1990). The starter inoculum of selected AM fungal spores was produced by using wheat as host plant through Funnel Technique of Menge & Timmer (1982) over 3 month period. The inoculum of *P. fluorescens* (MTCC NO. 103) was procured from the Institute of Microbial Technology, Chandigarh, India, and multiplied by using nutrient broth medium incubated for 48 hours at 32°C to obtain concentration of 1×10^9 colony forming units (cfu) mL^{-1}.

One month old saplings of *C. citratus* were procured from Chaudhary Devi Lal Herbal Nature Park, Yamuna Nagar, Haryana, India. In the case of control, saplings were planted after washing of roots. For treatment, roots were dipped in the cell suspension of *P. fluorescens* for up to 5 minutes before transplanting them in to earthen pots.

Soil used in the investigation site was sieved and mixed with sand in a ratio of 3:1 (soil: sand), subjected to steam sterilization at 121°C in autoclave for 1 h twice over a period of 3-days. Three saplings were transplanted in each of the earthen pots (size 25 × 25 × 10 cm) having sterilized soil and 10% w/w selected inoculums of AM fungi. Plants were grown under natural illumination, watered regularly and with eight treatments in a polyhouse.

The treatment details are as follows:

T_1 : Untreated control
T_2 : *Acaulospora laevis* (A)
T_3 : *Glomus mosseae* (G)
T_4 : *Pseudomonas fluorescens* (P)
T_5 : *Acaulospora laevis* + *Glomus mosseae* (A +G)
T_6 : *Acaulospora laevis*+ *Pseudomonas fluorescens* (A + P)
T_7 : *Glomus mosseae* + *Pseudomonas fluorescens* (G + P)
T_8 : *Glomus mosseae* + *Acaulospora laevis* + *Pseudomonas fluorescens* (G + A + P)

After 120-days growth period, the biometric observations were noted on plant height, root length and biomass respectively. The leaf area was measured by using leaf area meter (Systronics 211, Ahmedabad, India). The AM spores were quantified by the method suggested by Gerdmann & Nicolson (1963) and Gaur & Adholeya (1994). Root colonization was assessed by Rapid Clearing and Staining Method of Phillips & Hayman (1970) and root slide technique of Giovannetti & Mosse (1980).

The quantification of root colonization was done by the following formula:

Percentage root colonization = (Number of root segments colonized / Number of root segments studied) × 100

The biochemical parameters studied were chlorophyll (Arnon 1949), phosphorus content (Jackson 1973), phosphatase activity (Tabatabai & Bremner 1969) and oil (Santos *et al*. 2009). The yield of essential oil in percentage was calculated by using following formula:

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

The data was statistically analyzed by using 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 $P \leq 0.05$ on each of the significant variables for mean seperation.

Results and discussion

Analysis of experimental *C. citratus* plants showed that soil augmented with mixed bioinoculants recorded maximum improvement in vegetative growth of plants in comparison

to control plants (Table 1). G+A+P treatment was found to be dominant for increased plant height, shoot biomass in the form of fresh and dry weight, root length and root biomass in fresh and dry form. Similar type of improvement in vegetative growth parameters of plants was observed by Esha *et al*. (2018) by the application of phosphate solubilizing bacteria and AM fungi. An increment in growth parameters might be due to reduced level of pathogenic microorganisms in rhizosphere and improved nutritional status of plant is linked with increased surface area of roots for absorption of immobilised nutrients from soil system. *P. fluorescens* bacterium was found to assist plant growth by successful establishment of AM association with roots of plant that might be due to production of some plant growth hormones and secondary metabolites (Kurth *et al*. 2015). The plants inoculated with mixed AMF inoculums showed high AMF dependency for growth in comparison to control.

The increased leaf area (Table 2) and chlorophyll content (Table 3) in AM treated plant was closely linked with enhanced nutrients (P and Mg) and water absorption, which plays an important role in synthesis and functioning of photosynthetic pigments. An increased cytokinin activity and P absorption in AM inoculated plants act as causative factors behind increased leaf growth as well as leaf area (Jakobsen 1994; Saini *et al*. 2017).

After 4 months of inoculation with different bioinoculants, AM spore number and per cent mycorrhizal root colonization were highest in G+A+P treatment followed by G treatment and were positively correlated with each other (Table 2). The better AM root colonization

Table 1. Interactive effect of AM fungi and *P. fluorescens* on growth response of C*ymbopogon citratus* after 120 days

Treatment	Plant height (cm)	Shoot weight (g)		Root length (cm)	Root weight (g)	
		Fresh	Dry		Fresh	Dry
Control	43.10±6.84^{c}	13.33±2.58^{e}	1.34±0.18^{c}	08.74±0.69^{d}	1.49±0.17^{e}	0.35±0.18^{d}
A. laevis	66.48±6.29^{b}	18.96±1.96^{d}	2.07±0.25^{b}	16.26±1.19^{c}	3.77±0.26^{c}	1.17±0.33^{bc}
G. mosseae	69.50±9.36^{ab}	19.26±0.40^{d}	1.93±0.49^{b}	17.69±1.75^{c}	4.54±0.27^{ab}	1.60±0.13^{a}
P. fluorescens	72.95±1.12^{ab}	17.33±1.37^{d}	1.91±0.09^{b}	13.03±0.56^{cd}	2.71±0.07^{d}	1.01±0.03^{c}
A+G	73.83±5.02^{ab}	19.33±0.20^{d}	1.82±0.14^{bc}	23.76±3.62^{b}	4.79±0.51^{ab}	1.64±0.06^{a}
A+P	70.80±2.08^{ab}	23.96±0.46^{c}	1.85±0.16^{b}	27.66±2.83^{ab}	4.13±0.93^{bc}	1.34±0.31^{abc}
G+P	70.78±6.12^{ab}	27.73±1.10^{b}	2.10±0.35^{b}	27.60±2.49^{ab}	4.73±0.15^{ab}	1.45±0.14^{ab}
G+A+P	77.81±2.18^{a}	30.46±1.58^{a}	2.63±0.30^{a}	30.30±5.72^{a}	5.21±0.17^{a}	1.70±0.32^{a}
L.S.D (P≤0.05)	9.627	3.637	0.390	4.952	0.717	0.381
ANOVA F (7,16)	11.012	31.622	4.956	22.414	27.191	12.340

G†=*G. mosseae*; A=*A. laevis*; P=*P. fluorescens*; ‡=Each value is a mean of five replicates; ±=Standard deviation; AM=Arbuscular mycorrhizae; Values in columns followed by same letter are not significantly different;

Table 2. Interactive effect of AM fungi and *P. fluorescens* on leaf area, mycorrhization and phosphorus content in *C. citratus* after 120 days

Treatment	Leaf area (cm^2)	AM spore number/ 10 g of soil	AM root colonization (%)	Phosphorus content (%)	
				Shoot	Root
Control	13.33±4.16^{d}	18.36±1.85^{d}	18.33±0.40^{d}	0.117±0.002^{f}	0.150±0.017^{d}
A. laevis	15.73±2.96^{d}	78.60±6.90^{a}	75.86±9.36^{a}	0.191±0.006^{e}	0.378±0.081^{bc}
G. mosseae	49.36±0.75^{b}	78.80±1.68^{a}	78.16±1.16^{a}	0.240±0.008^{d}	0.539±0.092^{ab}
P. fluorescens	47.26±1.05^{b}	67.16±7.16^{b}	65.40±6.16^{b}	0.220±0.011^{d}	0.355±0.106^{c}
A+G	61.33±1.65^{a}	48.20±5.07^{c}	49.26±3.55^{c}	0.313±0.001^{c}	0.385±0.123^{bc}
A+P	41.70±2.34^{b}	50.03±4.78^{c}	56.36±1.95^{c}	0.431±0.003^{b}	0.531±0.098^{ab}
G+P	32.36±2.58^{c}	51.16±5.78^{c}	54.83±7.04^{c}	0.468±0.031^{a}	0.605±0.086^{a}
G+A+P	56.66±1.85^{a}	86.33±8.65^{a}	79.46±3.16^{a}	0.451±0.026^{ab}	0.661±0.062^{a}
L.S.D (P≤0.05)	1.760	9.912	8.725	0.027	0.154
ANOVA F(7,16)	26.789	46.160	47.885	217.434	10.365

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

was associated with better root architecture in terms of fibrous roots as a result of increased level of auxin due to AM fungi and phosphate solubilising bacteria inoculation (Prasad *et al*. 2012; Yadav *et al*. 2013b).

Estimation of P content revealed maximum percentage in G+P treated shoot, while G+A+P treatment was observed effective in roots. The results of present study coincide with the finding of Yadav *et al*. (2015), who reported increased absorptive surface area of the root as a result of increased fungal hyphal growth beyond the rhizospheric soil, which was ultimately linked with increased efficiency of nutrient absorption, especially slightly diffusing mineral ion like phosphorus. Mycorrhizal hyphae exploit the soil volume much more thoroughly for phosphorus than a non mycorrhizal root system. Both AM fungi and *Pseudomonas* spp, appeared to work synergistically and resulted in greater uptake of P than when each was inoculated alone (Saini *et al*. 2017; Esha *et al*. 2018). An increased level of P content was reported in *Pseudomonas* spp treated *Chrysanthemum indicum* (Prasad *et al*. 2012). There was higher root P content in AM treated plants which is probably due to more efficient uptake of available P and possible mineralization of organic P by AM treated plants, that help in uptake of P (León-Anzueto *et al*. 2011).

Phosphatase activity was noticeably higher in microbe's treated plants roots in comparison to untreated control along with direct relationship between mycorrhizal root colonization and phosphatase activity of *C. citratus* plants (Table 3). Highest phosphatase activity was observed in G+A+P treatment followed by A+P treatment. The phosphatase enzyme helps in conversion of bound P into soluble form in the rhizosphere and make it available to the plants through AM colonized roots (Yadav & Aggarwal 2014). Many researchers have reported an increase in phosphatase activity in mycorrhizae inoculated plants, which may be contributed by phosphatase activity of the intra-radical hyphae of mycorrhizal fungi. Bacteria may also

Table 3. Interactive effect of AM fungi and *P. fluorescens* on chlorophyll content, phosphatase activity and essential oil yield of *C. citratus* after 120 days

Treatment	Chlorophyll content (mg./gm. fresh wt.)			Phosphatase activity (IU g^{-1} FW)		Yield (%)
	Chl a	Chlb	Total chl	Acidic	Alkaline	
Control	0.63±0.36c	0.12±0.01^{b}	0.75±0.35^{b}	0.032±0.003^{d}	0.031±0.003^{c}	0.61±0.18^{d}
A. laevis	1.18±0.70^{abc}	0.97±0.68^{ab}	2.15±1.39^{ab}	0.041±0.003^{d}	0.038±0.002^{c}	1.56±0.26^{a}
G. mosseae	1.10±0.60^{abc}	0.99±0.62^{ab}	2.10±1.22^{ab}	0.103±0.019^{c}	0.040±0.001^{c}	1.44±0.29^{ab}
P. fluorescens	0.99±0.22^{abc}	0.93±0.52^{ab}	1.92±0.74^{ab}	0.033±0.009^{d}	0.037±0.003^{c}	0.80±0.14^{c}
A+G	0.89±0.45^{bc}	1.00±0.60^{ab}	1.89±1.04^{ab}	0.123±0.024^{bc}	0.118±0.004^{b}	0.96±0.28^{bc}
A+P	1.17±0.31^{abc}	1.26±0.47^{a}	2.43±0.59^{ab}	0.131±0.006^{b}	0.130±0.007^{b}	1.52±0.20^{a}
G+P	1.73±0.73^{ab}	1.68±0.34^{a}	3.42±0.85^{a}	0.123±0.014^{bc}	0.119±0.008^{b}	1.68±0.38^{a}
G+A+P	1.87±0.20^{a}	1.64±0.47^{a}	3.52±0.33^{a}	0.208±0.005^{a}	0.188±0.025^{a}	1.92±0.42^{a}
L.S.D (P≤0.05)	0.850	0.882	1.550	0.022	0.017	0.484
ANOVA F(7,16)	2.147	2.773	2.940	67.711	103.650	14.124

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

support AM symbiosis by releasing nonspecific phosphatases that dephosphorylate phosphor–ester or phosphoanhydride bonds in organic matter, C–P lyases that dissociate C–P bonds in organophophonates and phytases that release phosphorus from phytic acid (Idriss *et al*. 2002), which ultimately elevate P level in deficient soil.

Oil content was found to be minimum in untreated control plants and maximum in combination of bio-inoculants like G+A+P proceeded by dual combination than single. According to results G was found effective in association with other microorganisms whereas treatment A was effective in independent form**.** Similar trend in increment of oil yield on inoculation with bio-fertilizer was reported by several workers (Bahadori *et al*. 2013). The AM fungi and PGPR (Plant Growth Promoting Rhizobacteria) enhance and modify the biochemical contents like proline, essential oil and proteins in term of quantity as well as quality (Burni *et al*. 2013). These changes correspond to release of organic acid from microorganisms which lead to transfer of immobile phosphate to available form for the plants. This may support growth of roots in the soil that can take up more nutrients and help in increasing the synthesis of essential oil.

In the present study it can be concluded that the application of mycorrhizal fungi alone or in combination with *P. fluorescens* is considered to be beneficial for morphological parameters of lemon grass (*C. citratus*) in pot studies under controlled conditions in a polyhouse. The plants were found healthier with improved P content, photosynthetic pigments and phosphatase activity due to increased root colonization by

AM fungi assisted by bacterial inoculation. The symbiotic association was found significant in enhancement of essential oil yield up to 12% in combined inoculation of bio-fertilizers i.e. *G. mosseae* + *A. laevis* + *P. fluorescens* followed by G+P (10.20 %) > A+P (8.67%) > A+G (3.33%) treatment. Among single inoculation, *A. laevis* had maximum enhancement in essential oil yield i.e. 9.05% followed by *G. mosseae* (7.91 %), and *P. fluorescens* (1.81%). The beneficial effects are also corresponding to the water absorption due to increased surface area of roots along with secretion of some enzymes by inoculated microorganisms. However, further investigations at the field level are required to assess the efficacy of these micro-organisms under natural conditions.

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References

Arnon D I 1949 Copper enzymes in isolated chloroplasts, polyphenoxidase in *Beta vulgaris*. Plant Physiol. 24: 01–15.

Bahadori F, Sharifi Ashorabadi E, Mirza M, Matinizade M & Abdosi V 2013 Improved growth, essential oil yield and quality in Thymus daenensis celak on mycorrhizal and plant growth promoting rhizobacteria inoculation. Int. J. Agron. Plant Prod. 4: 3384–3391.

Burni T, Hussain F & Sharif M 2013 Effect of arbuscular mycorrhizal fungi on essential oils of two pharmaceutically important *Mentha* species in marginal soils. Pakistan J. Bot. 45: 293–296.

Fokom R, Adamoub S, Essonoc D, Ngwasiria D P, Eked P, Teugwa Moford C, Tchoumbougnanga F, Fekamd B F, Amvam Zollod P H, Nwagac D & Sharma A K 2019 Growth, essential oil content, chemical composition and antioxidant properties of lemongrass as affected by harvest period and arbuscular mycorrhizal fungi in field conditions. Ind. Crops Prod. 138: 11477.

Gaur A & Adholeya A 1994 Estimation of VAM spores in soil: a modified method. Mycorrhiza News 6: 10–11.

Gerdemann J W & Nicolson T H 1963 Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc. 46: 235–244.

Giovannetti M & Mosse B 1980 An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84: 489–500.

Idriss E E, Makarewicz O, Farouk A, Rosner K, Greiner R, Bochow H, Richter T & Borriss R 2002 Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB45 contributes to its plant-growth promoting effect. Microbiol. 148: 2097–2109.

Jackson M L 1973 Soil chemical analysis. Published by Printice Hall, New Delhi, pp.485.

Jakobsen I, Joner E J & Larsen J 1994 Hyphal phosphorus transport, a keystone to mycorrhizal enhancement of plant growth. In: Gianinazzi S & Shuepp H (Eds.), Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems (pp.133–146). Basel, Birkhauser, Switzerland.

Kurth F, Feldhahn L, Bonn M, Herrmann S, Buscot F & Tarkka M T 2015 Large scale transcriptome analysis reveals interplay between development of forest trees and a beneficial mycorrhiza helper bacterium. BMC Genomics 16: 658.

Leon- Anzeuto E, Abud-Archila M, Dendooven L, ventura-canseco L M C & Gutierrez-Miceli F A 2011 Effect of vermicompost, worm- bed leachate and arbuscular mycorrizal fungi on lemon grass (*Cymbopogon citrates* (DC) stapf.) growth and composition of its essential oil. Electron. J. Biotechnol. 14.

Lermen C, Cruz R M S, SouzaGonçalves C H, Cruz G L S, Silva G P A & Alberton O 2019 Growth of lemongrass (*Cymbopogon citratus* (DC) Stapf) inoculated with arbuscular mycorrhizal fungi (*Rhizophagus clarus* and *Claroideoglomus*

etunicatum) under contrasting phosphorus levels. Aust. J. Crop Sci. 13: 266–271.

Lermen C, Morelli F, Gazim Z C, Silva A P D, Alves J E G, Dragunski D C & Alberton O 2015 Essential oil content and chemical composition of *Cymbopogon citratus* inoculated with arbuscular mycorrhizal fungi under different levels of lead. Ind. Crops Prod. 76: 734–738.

Menge J A & Timmer L W 1982 Procedure for inoculation of plants with VAM in the laboratory, greenhouse and field. In: Schenck N C (Ed.), Methods and Principles of Mycorrhizal Research (p.59–67). American Phytopathology Society, St. Paul, Minn, USA.

Morone-Fortunato I & Avato P 2008 Plant development and synthesis of essential oils in micropropagated and mycorrhiza inoculated plants of *Origanum vulgare* L. ssp. Hirtum (Link) Ietswaart. Plant Cell Tissue Organ Cult. 93: 139–149.

Nambair V & Mehta H 2012 Potential functions of lemon grass (*Cymbopogon citratus*) in health and disease. Int. J. Pharm. Biol. Arch. 3: 1035–1043.

Ortiz R S, Marrero G V & Navarro A L T 2002 Instructivo técnico del cultivo de *Cymbopogon-citratus* (D.C) Stapf (caña santa). Rev. de Plantas Medicinales 7: 2.

Phillips J M & Hayman D S 1970 Improved procedures for clearing roots and staining parasitic and VAM fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55: 158–161.

Prasad K, Aggarwal A, Yadav K & Tanwar A 2012 Impact of different levels of superphosphate using arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* on *Chrysanthemum indicum* L. J. Soil Sci. Plant Nutr. 12: 451–462.

Robacker D C & Hendry L B 1977 Neral and geranial: components of the sex pheromone of the parasitic wasp, Itoplectis conquisitor. J. Chem. Ecol. 3: 563–577.

Sabannavar S J & Lakshman H C 2009 Effect of rock phosphate solubilisation using mycorrhizal fungi and phosphobacteria on two high yielding varieties of *Sesamum indicum* L. World J. Agric. Sci. 5: 470–479.

Saini I, Yadav K Esha & Aggarwal A 2017 Effect of bioinoculants on morphological and biochemical parameters of *Zinnia elegans* Jacq. J. Appl. Hort. 19: 167–172.

Santos A, Paduan R H, Gazin Z C, Jacomassi E, D´ıOliveira P S, Cortez D A G & Cortez L E R, 2009 Determina´ıc¸ o do rendimento e atividade antimicrobianado ãleo essencial de Cymbopogon citratus (DC.) Stapf em fun´ıc¸ o de sazonalidadee consorciamento. Rev. Bras. Farmacogn. 19: 436–441.

Smith S E & Read D J A P 2008 Mycorrhizal symbiosis. Academic Press, London.

Tabatabai M A & Bremner J M 1969 Use of *p*-nitrophenol for assay of soil phosphatase activity. Soil Biol. Biochem. 1: 301–307.

Yadav A & Aggarwal A 2014 Effect of dual inoculation of AM fungi and *Pseudomonas* with phosphorus fertilizer rates on growth performance, nutrient uptake and yield of Soybean. Res. 6: 05–11.

Yadav K, Aggarwal A & Singh N 2013b Arbuscular mycorrhizal fungi (AMF) induced acclimatization and growth enhancement of *Glycyrrhiza glabra* L. - A potential medicinal plant. Agric. Res. 2: 43–47.

Yadav K, Singh N & Aggarwal A 2012 Arbuscular mycorrhizal technology for the growth enhancement of micropropagated *Spilanthes acmella* Murr. Plant Prot. Sci. 48: 31–36.

Yadav A, Yadav K & Aggarwal A 2015 Impact of arbuscular mycorrhizal fungi with *Trichoderma viride* and *Pseudomonas fluorescens* on growth, yield and oil content in *Helianthus annuus* L. J. Ess. Oil Res.18: 444–454.