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Synergistic effect of Arbuscular Mycorrhizal fungi and *Azotobacter chroococcum* on the vegetative growth and herbage yield of *Cymbopogon citratus* (DC.)

Ambrish Kumar, Deeksha Pathak, Shalini Jadon, Shivam Parmar and Saroj Singh Chahar*

Department of Botany, R.B.S. College, Agra - 282002 (India)
(Affiliation with Dr. Bhimrao Ambedkar University, Agra - 282004 (India)
***Corresponding author:** Saroj Singh Chahar, Associate Professor, Department of Botany, R.B.S. College, Agra, (India)
E-Mail: - ambrish.rbs@gmail.com

Abstract

The findings have shown that soil microorganisms have a major impact on soil fertility and plant growth. Symbiotic arbuscular mycorrhizal fungi (AM fungi) play a vital role in soil microbial flora. The AM fungi have a mutualistic connection with the host plant, enhancing its development and nutrient intake. *Azotobacter chroococcum*, a bacterium that fixes nitrogen and mobilizes phosphate, and mycorrhizal fungi can boost plant nutrition and hence act as biofertilizers in my test crops. An experiment was carried out to determine the growth promotion of *Cymbopogon citratus* (DC.) after single and dual inoculation with AM fungi and *Azotobacter chroococcum*. The effects on the length and width of leaves, fresh shoot weight, dry shoot weight, and number of tillers at the 180th day was investigated. On the 180th day, inoculated plants with *Azotobacter chroococcum* and AM fungi produced more favorable results than plants fertilized with chemical fertilizers (N, P and K). During the 2021 and 2022 seasons, however, the combination treatments of N and P with bio-fertilizers led to a significant increase in plant height, width of leaves, fresh and dry shoot weight, and number of tillers, when compared to the control. The findings showed that inoculating *A. chroococcum* and AM fungi together improved the vegetative growth of lemongrass. Therefore, above findings revealed that nitrogen fixation, phosphate solubilization, and mineralization were beneficial to plant growth and development.

Key words : *Azotobacter chroococcum*, Lemongrass, microorganisms, bio-fertilizers, agriculture, inoculation, and mycorrhiza.

Cymbopogon citratus, a monocotyledon which belongs to the Poaceae family[13], is one of the most promising essential oil crops. It is grown over several regions of Africa and South East Asia, both tropical and subtropical. The plant, popularly known as lemon grass (*Cymbopogon citratus*) is indigenous to Sri Lanka, India, and Pakistan[22]. It's a perennial grass with multiple stiff stems that grow from a small, rhizomatous root. The leaves are about 50 cm long and 1.5 cm wide, and they are extensively utilized to produce valuable essential oils, cellulose, and paper[33]. It has significant biological potential due to the presence of several types of aldehydes, terpenes, phenolic, and other antibacterial compounds[21]. The leaves contain 0.25-0.35% essential oil, where Citral make up the majority component (80-86%)[1]. Interestingly, Citral has been identified as the main component responsible for lemongrass oil's antibacterial properties[11].

When we adopt sustainable agriculture practices, arbuscular mycorrhizal fungi (AMF) may be the most promising fungal biofertilizers, especially for stress, nutrition, and disease management[12]. Arbuscular mycorrhizal fungi are obligate symbionts of the Glomeromycota phylum that form mutualistic symbiotic interactions with more than 80% of land plant species, including several crops[25]. They provide the host plant with mineral nutrients and water in exchange for photosynthetic products. Arbuscular mycorrhizal fungus helps to retain nutrients in the soil and lower the risk of groundwater contamination. Sun and Shahrajabian[32] claimed that the final methodology enhances plant and water intake by enlarging root structures, improving resilience to biotic and abiotic stresses, and increasing plant antioxidant status. Several authors have proven the role of organic fertilizer in increasing vegetative growth and essential oil in a range of medicinal and aromatic plants, including lemon grass[31]. Several organic farming practices have been used to increase plant yield and essential oil in lemongrass. Many researchers, notably Mona (2006) on *Plantago afra* L, Khalid *et al*.,[16] on *Ocimum basilicum*, and Abdullah *et al*.[3] on *Rosmarinus officinalis*, discovered that biofertilizers increased growth, yield, and chemical composition. Biomass yield rose by 3–10% after inoculating lemongrass with arbuscular mycorrhizal fungus (AMF), such as *Rhizophagus mosseae* and *Rhizophagus fasciculatum*[10].

Azotobacter chroococcum, a Gram-negative bacterium[7] from the proteobacteria family Azotobacteraceae, is a cohesive group of aerobic, free-living diazotrophs capable of fixing atmospheric nitrogen in nitrogen-free or nitrogen-poor environments by utilizing organic carbon compounds as an energy source[28]. *Azotobacter chroococcum* is hypothesized to possess various characteristics that contribute to its beneficial effects on related plants. The preceding research review shows that regardless of the amount of nitrogen fertilizer applied to crops, using *Azotobacter* as a biofertilizer, either alone or in conjunction with AMF, increases overall growth of crops[6]. The advantages of employing bio inoculants include higher plant growth and production, improved quality and crop uniformity, reduced 'N' and 'P' micronutrient fertilizer requirements, and lower losses due to environmental difficulties and diseases[19]. Many scientists now regard biofertilizers as a

viable alternative, especially in developing countries. Rhizosphere organisms' behaviors have been well reported in non-leguminous plants such as tropical grasses[35]. These organisms can influence their host plant through a variety of mechanisms, including nitrogen fixation, the production of growth-promoting compounds or organic acids, enhanced nutrient uptake, and pathogen protection[15].

The primary goal of the current study was to increase the usage of various biofertilizers with or without varying quantities of urea and phosphorous in the growth of *Cymbopogon citratus*.

The average yearly temperature in Agra ranged from 47°F to 105°F. The relative humidity was 70%. The soil pH in the experimental field was 7.81, with an electrical conductivity (EC) of 0.663 ds/m. The investigation found that the soil nitrogen level was low, 0.72g/kg (Kjeldhal method). The soil organic matter concentration was also very low, 0.32% by wet oxidation method. The study looked at the effects of single and dual inoculation of arbuscular mycorrhizal fungi (AMF only), *Azotobacter* (*A. chroococcum* only), and arbuscular mycorrhizal fungi + *Azotobacter* (AMF+ *A. chroococcum* both), including a blend, on *Cymbopogon citratus* (DC.)

Plant material and Microbial biofertilizers:

Uniform Seedlings (slips) of *Cymbopogon citratus* (30 cm in length of each) were procured from CIMAP, a CSIR institute (Central Institute of Medicinal and Aromatic Plants, Lucknow, U.P.) The mixture of biofertilizers of AM Fungi and pure culture of *Azotobacter chroococcum* were procured from RBS Community College, Bichpuri, Agra, (U.P.). Mixed spores of AM Fungi were mixed with soil and then added to each treated plant at a rate of 50g /plant as each gram included approximately 500 spore /g inoculum. Active strain of *Azotobacter chroococcum* was grown on modified Ashby's medium[2]. It was inoculated in 250 ml conical flasks that contained 100 ml Ashby's medium for 5 days at 28±2 ^{o}C, then enriched in the same medium and incubated for 7 days to reach 106 cfu / ml and then added to each plant soil at a rate of 10 ml inoculum/plant according to the decided treatments. A small dose of biofertilizers is sufficient because each gram of carrier of biofertilizers contains at least 10 million viable cells of a specific strain[5].

Experimental trials:

Cymbopogon citratus seedlings (slips) of around 30 cm in length were planted in plots in the first week of March 2021, after being properly inoculated with *Azotobacter chroococcum* and Arbuscular Mycorrhizal Fungi. The experiment was designed using a complete randomized blocks design, with each plot measuring 2×1.5 m. Individually transplanted slips were spaced 60 cm apart, with rows 40 cm apart. Each allotment has around ten plants. The treatments were then reproduced three times (30 plots in total). Irrigation began shortly after transplantation and maintained as needed to achieve optimal growth. This experiment took place in the Botanical Garden of the Department of Botany, R.B.S. College Agra, during the 2021 and 2022 seasons (Tables 2-5). The treatments consisted of nine distinct organic and inorganic fertilizer combinations (Table-1), as well as a control.

Table-1. Application of biofertilizers and chemical fertilizers (N, P, and K) under single, dual and blended inoculation were studied as per the following treatments:

CONTROL A	Without any addition of fertilizers
CONTROL B	With normal doze (100%) of chemical fertilizers (N, P & K)
C	Nitrogen 50% (1/2urea)
D	Phosphorous 50% (1/2 single super phosphate)
E	*Azotobacter chroococcum* (mono inoculation)
F	Arbuscular mycorrhizal fungi (AMF) (mono inoculation)
G	Arbuscular mycorrhizal fungi (AMF) + *Azotobacter* (dual inoculation)
H	Nitrogen 50 % + *Azotobacter chroococcum* (blended)
I	Phosphorous 50 % + AMF (blended)
J	Nitrogen 50 % + Phosphorous 50 % + *Azotobacter* + AMF (blended)

The plots included biofertilizer levels as well as NPK/Biofertilizer treatments such as 100% NPK, 50% N (urea), 50% P (single super phosphate), *Azotobacter* (mono inoculation), AMF (mono inoculation), AMF + *Azotobacter* (dual inoculation), Nitrogen (urea 50%) + *Azotobacter* (blended), Phosphorous 50% + AMF (blended), and nitrogen 50% + phosphorous 50% + *Azotobacter* + AMF (Table-1).

Growth attributes :

The following parameters were recorded viz. plant height, leaf area, number of leaves, wet and dry weight of leaves, number of tillers per plant, and fresh and dry weight of herbage of *Cymbopogon citratus* (g/plant).

Harvesting (Cuttings) :

Cymbopogon citratus plants were picked twice using a sickle 20 cm above ground level. The first cutting took place six months after cultivation, in September, and the second, three months later, in December. Following harvest, fresh and dry weights were determined. These dried and divided leaves were stored individually in polybags at room temperature in the laboratory until they were analyzed for essential oils.

Statistical analysis of growth attributes :

Data for each cut included plant height (cm), leaf area (cm), number of leaves, fresh and dried weights of leaves/plant, and number of tillers per plant. The obtained data were statistically analyzed for ANOVA and it was used to compare the means of treatments.

However, the soil health of the experimental site was very poor and challenging but the experiment successfully revealed and outcome of this problem with the help of selected biofertilizers. The growth characteristics of *Cymbopogon citratus* were as follows-

Effect of different fertilizers and biofertilizers on the growth parameters of Lemongrass plants:

The synergistic or additive interactions between A M Fungi and *Azotobacter chroococcum* can be linked to a number of mechanisms, which are listed below. Crops fared better following fertilizer application compared to the control treatment[9]. When the various fertilizer formulations were examined, during the two cuts in the both seasons, it is noticed from data that NPK full dose resulted in significant increases values and the 'J' fertilizer mixture, which contained 50% N, 50% P, AMF and *Azotobacter chroococcum*, produced the most leaf growth (Table-2 & 3). This is congruent with the findings of Harb and Eltatawy[14], who discovered that plants treated with both inorganic and bio-fertilizers had considerably more leaves per clump of lemongrass. Throughout the study, the number of tillers per plant increased with plant age in each treatment[8]. The highest rate of tiller growth was observed three to four months after planting. The 'J' fertilizer mixture led to a higher average number of tillers per plant (29.43) six months after planting (Table 4 & 5). Similar findings were reported by Sharma *et al*.,[29], who showed that plants cultivated without fertilizer had significantly fewer tillers per clump[34]. Plant height increased at a rapid pace for four months before sowing during the study period[18]. Table 5 demonstrates that several fertilizer combinations had a significant effect on plant height. Sharma *et al.,*[29] discovered significantly lower plant heights in control treatments, which validates the current results. Malgioglio *et al*.,[20] discovered an increase in plant height with increased N application and hypothesized that this was due to nitrogen, one of the important nutrients required for plant growth.

Following six months of inoculation with various combinations, root colonization reached its maximum (32.16 cm) in the J (Nitrogen 50% + Phosphorous 50% + *Azotobacter* + AMF) treatment in the second cutting of the second season compared to the untreated control A (10.50 cm) of the first cutting of the first season. There was also a direct correlation between phosphatase activity of Cymbopogon citratus plants and mycorrhizal root colonization. Because of the higher level of auxin caused by the inoculation of phosphate-solubilizing bacteria and AM fungus, improved AM root colonization was linked to better root architecture in terms of fibrous roots[24]. The current study's findings align with those of Yadav *et al*.[36], who observed that fungal hyphal growth beyond the rhizospheric soil increased the absorptive surface area of the root.

This, in turn, was linked to increased nutrient absorption efficiency, particularly for minerals like phosphorus that diffuse slightly. Compared to a non-mycorrhizal root system, mycorrhizal hyphae use the soil volume for phosphorus far more extensively[26]. The fertilizer mixture J, which was blended with chemical fertilizers and optimal for optimum assimilation of nitrogen and phosphorus, contains Azotobacter + AMF + 50% Urea + 50% Phosphate.

Table-2. Effect of Bio-fertilizer and chemical fertilizers (Nitrogen or Phosphorus) on Plant Height, Leaf Area, No. of Leaves, fresh wt. of Leaves, Dry wt. of Leaves, and No. of Tillers of *Cymbopogon citratus*:

TABLE 2. FIRST YEAR (2021)

FIRST CUTTING

Treatments/ Parameters	Plant Height (cm)	No. of Tillers	No. of Leaves	Leaf area (cm^2)	Fresh Wt. of Leaves	Dry Wt. of Leaves	Root length (cm)
Control A	58.49	8.20	8.53	132.50	225.69	60.70	10.50
Control B	84.71	14.7	10.20	186.60	247.97	70.36	27.65
N 50%	59.69	10.3	8.80	169.20	248.81	67.12	27.24
P 50%	60.76	11.5	10.30	180.43	302,73	71.23	15.26
Azotobacter	62.93	12.1	9.70	177.70	326.51	77.11	16.02
AMF	66.30	13.4	12.0	192.16	269.74	80.26	13.18
Azo. + AMF	70.44	13.5	12.60	277.33	289.80	85.13	17.51
N + Azo.	78.21	14.6	11.60	288.30	408.87	87.34	27.46
P + AMF	86.11	15.5	10.36	297.90	419.91	91.26	18.62
50%N+50%P+ Azo+AMF	90.35	29.1	15.50	306.90	499.15	128.85	30.52
SE ±	0.25	0.18	0.07	0.39	0.36	0.34	0.16
CD at 5%	0.75	0.55	0.22	1.15	1.06	1.00	0.47
CD at 1%	1.02	0.75	0.30	1.58	1.46	1.37	0.65

TABLE 3. FIRST YEAR (2021)

SECOND CUTTING

Treatments/ Parameters	Plant Height (cm)	No. of Tillers	No. of Leaves	Leaf area (cm^2)	Fresh Wt. of Leaves	Dry Wt. of Leaves	Root length (cm)
Control A	59.73	8.33	8.53	131.13	226.22	60.79	10.63
Control B	85.87	14.78	10.23	183.70	248.43	70.45	27.72
N 50%	60.88	10.40	8.80	169.03	249.41	67.28	27.29
P 50%	61.59	11.65	10.37	177.50	302.47	71.28	15.32
Azo.	63.15	12.20	9.77	174.97	326.33	77.19	16.23
AMF	67.65	13.57	12.10	189.03	270.03	80.33	13.29
Azo. + AMF	70.84	13.55	12.67	274.13	289.99	85.26	17.62
N + Azo.	79.30	14.72	11.67	282.83	409.49	87.46	27.62
P + AMF	87.97	15.63	10.37	288.03	419.91	91.29	18.71
50%N+50%P+ Azo+AMF	91.49	29.2	15.60	306.93	498.90	129.01	30.65
SE ±	0.43	0.18	0.06	1.35	0.26	0.33	0.16
CD at 5%	1.29	0.53	0.17	4.01	0.77	0.97	0.49
CD at 1%	1.77	0.73	0.23	5.49	1.06	1.33	0.67

TABLE 4. SECOND YEAR (2022)

FIRST CUTTING

Treatments/ Parameters	Plant Height (cm)	No. of Tillers	No. of Leaves	Leaf area (cm^2)	Fresh Wt. of Leaves	Dry Wt. of Leaves	Root length (cm)
Control A	60.79	8.53	8.63	134.30	226.79	61.81	11.46
Control B	86.67	14.87	10.30	188.57	248.60	71.48	28.50
N 50%	61.81	10.50	8.83	171.13	249.82	68.23	28.04
P 50%	63.80	11.73	10.43	182.17	304.01	72.04	16.12
Azo.	65.61	12.30	9.80	179.63	327.52	78.19	17.26
AMF	68.73	13.67	12.10	194.13	270.88	81.04	14.27
Azo. + AMF	72.89	13.63	12.70	279.43	290.56	86.24	18.58
N + Azo.	80.55	14.83	11.70	290.03	409.80	88.16	28.61
P + AMF	89.43	15.70	10.30	299.00	420.83	92.06	19.46
50%N+50%P+ Azo+AMF	92.34	29.30	15.63	308.77	499.51	129.96	31.67
SE ±	0.17	0.18	0.09	0.47	0.40	0.34	0.25
CD at 5%	0.50	0.55	0.28	1.41	1.19	1.02	0.74
CD at 1%	0.69	0.75	0.38	1.93	1.63	1.40	1.02

TABLE 5. SECOND YEAR (2022)

SECOND CUTTING

Treatments/ Parameters	Plant Height (cm)	No. of Tillers	No. of Leaves	Leaf area (cm^2)	Fresh Wt. of Leaves	Dry Wt. of Leaves	Root length (cm)
Control A	61.92	8.63	8.73	133.19	227.23	63.81	11.58
Control B	87.82	14.95	10.40	186.87	250.09	72.58	28.75
N 50%	62.93	10.63	8.93	169.78	251.47	69.41	28.27
P 50%	63.65	11.82	10.53	181.34	305.52	72.97	16.43
Azo.	66.80	12.47	9.90	178.59	329.50	78.95	17.96
AMF	69.67	13.36	12.20	192.24	273.80	82.69	14.63
Azo. + AMF	73.69	13.73	12.80	277.01	292.48	86.99	18.79
N + Azo.	81.67	14.93	11.82	288.51	411.49	89.19	29.18
P + AMF	90.46	15.79	10.57	297.38	423.13	93.31	19.70
50%N+50%P+ Azo+AMF	93.69	29.43	15.77	307.34	500.85	131.97	32.13
SE ±	0.32	0.18	0.07	0.69	0.57	0.36	0.32
CD at 5%	0.95	0.52	0.22	2.05	1.68	1.07	0.96
CD at 1%	1.30	0.72	0.30	2.81	2.30	1.47	1.32

Cymbopogon citratus leaves are the most economically valuable part, and they are commonly utilized to extract essential oils. Fresh herbage yield and dry yield are the most important oil yield-contributing factors in lemon grass production[30,37]. Comparison of both the season research demonstrates that the control treatment resulted in significantly lower herbage yield (225.69, 226.22, 226.79, 227.23g/plant) and dry matter yield (60.70, 60.79, 61.81, 63.81g/plant) in all the cuttings. However, fresh (499.15, 498.90, 499.51, 500.85g/plant) and dry wt. (128.85, 129.01, 129.96, 131.97g/plant) plant yields were higher in the 'J' fertilizer mixture (50% N, 50% P, AMF, and *Azotobacter*) than rest of the other fertilizer mixtures. Similarly, Amirnia *et al*.[4] discovered that biofertilizer application considerably increased herbage yield when compared to the control. Santoyo *et al*.,[27] reported comparable findings, stating that organic and inorganic fertilizers resulted in the maximum dry matter content in lemongrass. Kilam *et al*.,[17] discovered that as nitrogen levels rose, dry matter buildup increased dramatically. NPK fertilizers are more efficient than organic fertilizers in supplying N, P, and K in the short term; however, biofertilizers have an advantage in supplying other micronutrient components that are not found in NPK fertilizers in the long run, as well as their consistent performance.

The current study discovered that using chemical fertilizers in conjunction with biofertilizer application had a significant impact on crop development, leaf harvest, and dry herbage of *Cymbopogon citratus* (DC.). A combination of inorganic fertilizer and biofertilizers (AMF: Azotobacter: Urea 50%: SSP 50% per plant) was the most promising fertilizer combination for crop growth and development. The effects of inoculation with arbuscular mycorrhizal fungus (mixtures of AMF) on *Cymbopogon citratus* root colonization, plant growth, and nutrient acquisition were studied in the field. AMF and *Azotobacter chroococcum* inoculation significantly increased root colonization, plant height, no of tillers, fresh herbage, and dry matter yield as compared to non-inoculated crops. Above biofertilizers inoculation significantly increased N, P, and K uptake by lemongrass shoot tissues, with P having the largest increase. We believe that inoculating lemon grass with AMF and *Azotobacter chroococcum* could significantly improve root colonization, growth, and nutrient uptake, enabling for commercial production in the field. By these tests, it was discovered that using a large number of biofertilizers with 50% or less inorganic fertilizers yields excellent biomass production results.

As a result, microorganism-based biofertilizers have the potential to replace chemical fertilizers, both in terms of agricultural productivity and environmental health. Many biofertilizers are simpler to use and less expensive than chemical fertilizers.

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