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Effects of consortium of Arbuscular Mycorrhizal Fungi and *Bacillus lehensis* strain MLB2 on *Ocimum sanctum* grown under NaF salinity stress

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Abstract
The aim of the present study was to analyse the synergistic effect of both, Arbuscular Mycorrhizal fungi (AMF) and *Bacillus lehensis*, on the growth of *Ocimum sanctum* grown under 40 ppm of Sodium fluoride stress. *Glomus mossae* and *Bacillus lehensis* strain MLB2 increased plant weight by 41% and 9% respectively as compared to control. Consortium of *Glomus mossae* and *Bacillus lehensis* strain MLB2 resulted in 44% increase in Fresh herbage yield and 42% in leaf fresh weight of *Ocimum sanctum.* Plant growth promoting rhizobacteria when grown in association with various mycorrhizal fungi showed a remarkable increase in plant height, leaf fresh weight, and total fresh biomass. Best consortium result with *Bacillus lehensis* was shown by *G. mossae* followed by G. *fasciculatum* G. *aggregatum* and G.*intraradices*. Inoculation of tulsi seedlings with mycorrhizal fungi and *Bacillus lehensis* increased the fluoride tolerance level of the herb.

Keywords: Arbuscular Mycorrhizal Fungi, *Bacillus lehensis*, Consortium, salinity stress, Sodium fluoride, *Ocimum sanctum*

Introduction
Salt stress in soil or water is one of the major stresses in arid and semi-arid regions which severely limit plant growth and productivity. Fluoride stress has deleterious effects on plant growth which includes (1.) water stress, 2.) Nutritional imbalance 3.) Salt-stress or 4.) combination of above factors. Fluoride accumulation, in even low concentrations can cause an abnormal change in biochemical and physiological parameters in plants and animals. In higher concentrations, it causes dental and skeletal fluorosis in humans (Lakshmi 2013) [8]. Therefore, development of salt-tolerant varieties is one of the strategies to increase production in salinity-stress prone areas.
Ocimum sanctum is a medicinal and aromatic plant (MAP), belonging to family *Lamiaceae* (Grayer *et al.* 1996) [6]. It is an annual herb native to India and other parts of Asia (Klimankova *et al*. 2008) [7]. The use of plant parts like root, stem and leaves has been maintained traditionally (Leonti *et al.* 2003). Mycorrhizal fungi are found everywhere. These symbionts attach and become part of the plant. AM fungi enhance the nutrient uptake and crop yield by solubilizing phosphate. If there is high concentration of phosphorous in the soil, mycorrhiza never die rather they control the phosphorus content of the soil (Smith *et al.* 1997) [12].
Bacillus lehensis strain MLB2 is a gram positive, alkali-tolerant and endospore forming bacteria (Ghosh *et al.* 2007) [15]. Fluorine is the most electronegative atom, and therefore has the ability to make strong hydrogen bonds. Fluoride accumulation, in even low concentrations can cause an abnormal change in biochemical and physiological parameters in plants and animals. In higher concentrations, it causes dental and skeletal fluorosis in humans (Lakshmi 2013) [8].

Material and Method
Sample collection
A bacterium, *Bacillus lehensis* strain MLB2, was isolated from the fluoride affected soil of Sirsahakhera region of Unnao district in Uttar Pradesh, India and was tested against different Sodium fluoride (NaF) concentrations: 100ppm, 200ppm, 300ppm, 400ppm and 500ppm.

Experimental site
The experimental site, Lucknow (Uttar Pradesh), with a warm humid subtropical climate is situated in the north-eastern part of Uttar Pradesh, India. Latitude: 26°50′21″ N, Longitude: 80°55′23″ E and Elevation above sea level: 126 m = 413 ft. The average annual rainfall of this area is 313 mm, which is evenly distributed from June to October and August is the wettest month of the year. The average temperature ranges from 26°C to 39°C and actual temperature ranges from 29°C to 47°C. The relative humidity fluctuate between 34 % and 92%.

Seed treatment
Seeds of *Ocimum sanctum* were obtained from the National Gene Bank for Medicinal and Aromatic Plants at the CSIR-Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow, India. *Ocimum sanctum* seeds were surface sterilized with 10% NaOCl for 5 minutes and properly rinsed with distilled water for 5 times before sowing.

Experimental set-up
Seeds of *O. sanctum* were sown in the polyethylene bags filled with sterile soil. 15 days old seedlings were then transferred to the earthen pots containing 1kg soil by using *5* g of inoculum of different AM fungi per seedling respectively placed at 5cm depth in pots. The seedlings were dipped in the bacterial inoculum solution of *Bacillus lehensis* for 30 min and were transferred to the pots having AM Fungi. The experimental set up was in completely randomized block design with three replicates of each, i.e. control and treatments in a glass house. The inoculum of different glomus fungi (*Glomus mossae*, *Glomus fasciculatum, Glomus intraradices and Glomus*

aggregatum) were obtained from CSIR-Central institute of medicinal and aromatic plants, Lucknow (U.P)

Determination of biochemical parameters

After 2 months, the crop was harvested to determine various physical and biochemical parameters.

Spore count and percent root colonisation

The spores produced by G. *mossae*, G. *fasciculatum* G. *aggregatum* and G.*intraradices* were counted by following the wet sieving and decanting method (Gerdemann & Nicolson, 1963).

Percentage colonization of roots by arbuscules and vesicles was done by McGonigle *et al*. 1990 method.

Statistical analysis

The collected data was subjected to statistical analysis for analysis of variance method (ANOVA), suitable to completely randomized design (CRD) for pot experiment with the help of software ASSISTAT 7.7 beta version. Microsoft excel was used for calculating Standard deviation and Standard error. The means were calculated using Duncan's multiple range tests under a significance level of $P \leq 0.05$.

Result

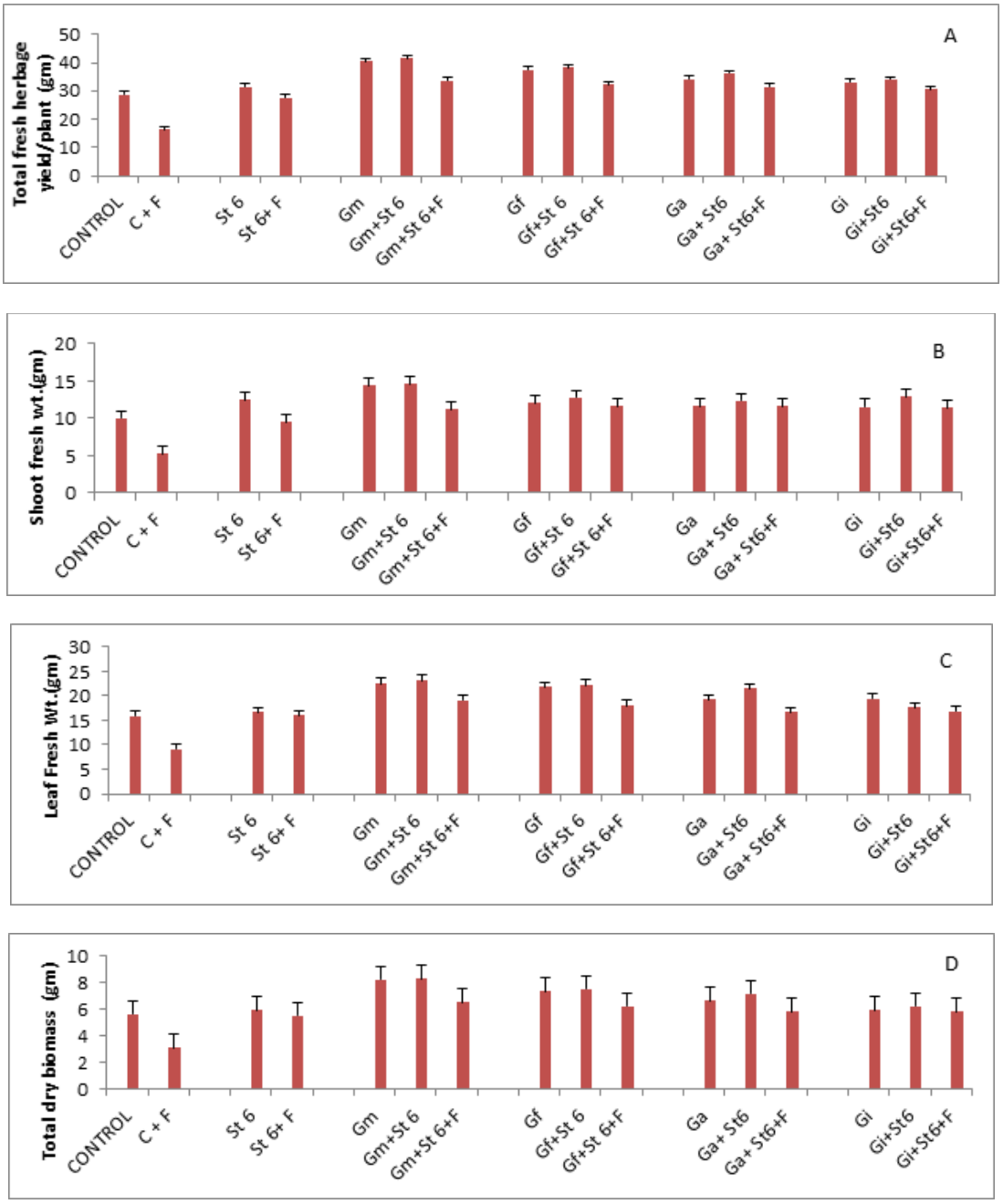

Figure: Histograms showing effect of various AM fungi and *B. lehensis* strain MLB2 on A.)Total fresh herbage yield/plant, B.)Shoot fresh weight, C.)Leaf fresh weight and D.) Total dry biomass of *Ocimum sanctum.* Where F: Sodium fluoride, St 6: *Bacillus lehensis* strain MLB2, Gm: *Glomus mosseae*, Ga: *Glomus aggregatum*, Gf: *Glomus fasciculatum*, Gi: *Glomus intraradices.*

Table 1: Effects of various treatments involving different AMF and *Bacillus lehensis* strain MLB2 on *Ocimum sanctum* grown under NaF stress.

S. No	Treatments	Total fresh herbage/ plant (gm)	Shoot fresh weight(gm)	Leaf fresh weight (gm)	Total dry weight(gm)
1	Control	28.74h	10.116e	16.226fg	5.659fg
2	C + F	16.34i	5.216 f	9.133h	3.112h
3	St 6	31.46fg	12.502bc	16.666ef	5.931ef
4	St 6 + F	27.62h	9.421e	16.052g	5.514g
5	Gm	40.56a	14.402a	22.341ab	8.219a
6	Gf	37.52bc	12.102bc	22.178ab	7.514b
7	Ga	36.22c	11.640cd	21.479b	7.111bc
8	Gi	34.03d	11.579cd	17.058c	6.202de
9	Gm+St 6	41.48a	14.618a	23.145a	8.374a
10	Gm+St 6+F	33.61de	11.201d	19.124cd	6.541d
11	Gf+St 6	38.13b	12.769b	22.816ab	7.285b
12	Gf+St 6+F	32.16ef	11.621cd	18.018de	6.201de
13	Ga+St 6	34.22d	11.649cd	19.257cd	6.669cd
14	Ga+ St 6+F	31.41fg	12.378bc	16.689ef	5.845ef
15	Gi+St6	33.06de	12.918b	17.610ef	5.954ef
16	Gi+St 6+F	30.64g	11.374cd	16.814ef	5.835ef

*Values denoted by same letter are not significantly different at $P<0.05$ level

Where F: Sodium fluoride, St 6: *Bacillus lehensis* strain MLB2, Gm: *Glomus mosseae*, Ga: *Glomus aggregatum*, Gf: *Glomus fasciculatum*, Gi: *Glomus intraradices*.

Discussion

Sodium fluoride has inhibitory effect on plant growth. Neutral to alkaline pH favors germination of *G. mossae*. Plants inoculated with AM fungi cultivated under saline conditions resulted in an increase in root length, fresh and dry weights of shoot and increased photosynthesis (Shhekoofeh and Sepideh 2011). Total fresh herbage yield increased up to 44% and leaf fresh weight increased to 42% in plants treated with consortium as compared to control. Synergistic effect of AM fungi and *B.lehensis* showed a remarkable coping effect of the herb against fluoride stress. The best coping effect was shown by *G.mossae* followed by *G.fasiculatum*, *G.aggregatum* and *G. intraradices* respectively.

Conclusion

Being a medicinally important plant, shoot of *Ocimum sanctum* is rich in essential oil which are pharmaceutically useful and as a result of increasing interest in natural herbs and medicines, more effort is now needed to develop better quality and quantity of essential oil herbs. By the inoculation of basil seedlings with Arbuscular mycorrhizal fungi, plant height and fresh biomass was increased as compared to control. Synergistic effect of AM fungi (AMF) inoculation with *Bacillus lehensis* under fluoride stress resulted in increase in leaf & shoot fresh weights by coping up with stress effects of fluoride. In this way, AM fungi and *Bacillus lehensis* used synergistically can prove to be a panacea for better yielding of natural herbs in stress conditions.

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