International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Synergistic Effects of AMF and *Bacilluslehensis* Strain MLB2 on *Ocimum sanctum* Grown under Fluoride Stress

Nidhi Yadav[1], Munna Singh[2]

[1]Department of Botany, University of Lucknow, Lucknow- 226007(U.P), India

[2]Vice-Chancellor, CSA agricultural University, Kanpur (U.P), India

Abstract: ***The aim of the present study was to notice the synergistic effect of both, Arbuscular Mycorrhizal fungi (AMF) and Bacillus lehensis, on the growth of Ocimum sanctumvar. CIM-AYU grown under 40 ppm of Sodium fluoride stress. Glomus mossae and Bacillus lehensis strain MLB2 increased plant weight by 41% and 9% respectively. Consortium of Glomus mossaeandBacillus lehensisstrain MLB2 resulted in44% increase in Fresh herbage yield of Ocimum sanctumas compared to control. 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 was shown by G.mossae followed by G. fasciculatum G. aggregatum and G.intraradices. Inoculation of tulsi seedlings with mycorrhizal fungi and Bacillus lehens is increased the fluoride tolerance level of the herb.***

Keywords: Arbuscular Mycorrhizal Fungi, Consortium, Sodium fluoride,*Ocimum sanctum*

1.Introduction

From ancient time, Tulsi is used as a traditional remedy for wound healing and microbial infections.*Ocimum sanctum* is a medicinal and aromatic plant (MAP), belonging to family*Lamiaceae* (Grayer *et al.* 1996). It is an annual herb native to India and other parts of Asia (Klimankova *et al.* 2008). The use of plant parts likeroot, stem and leaves has been maintained traditionally (Leonti et al. 2003). Medicinal herbs have been used from pre-historic times (Dragland *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).

*Bacillus lehensis*strainMLB2 is a gram positive, alkali-tolerantand endospore forming bacteria (Ghosh *et al.* 2007). Fluorine isthe 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).

2.Material and Method

Sample Collection

A bacterium, *Bacillus lehensis*strainMLB2, 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 washarvested to determine the physical parameters, chlorophyll contentand enzymatic activity.

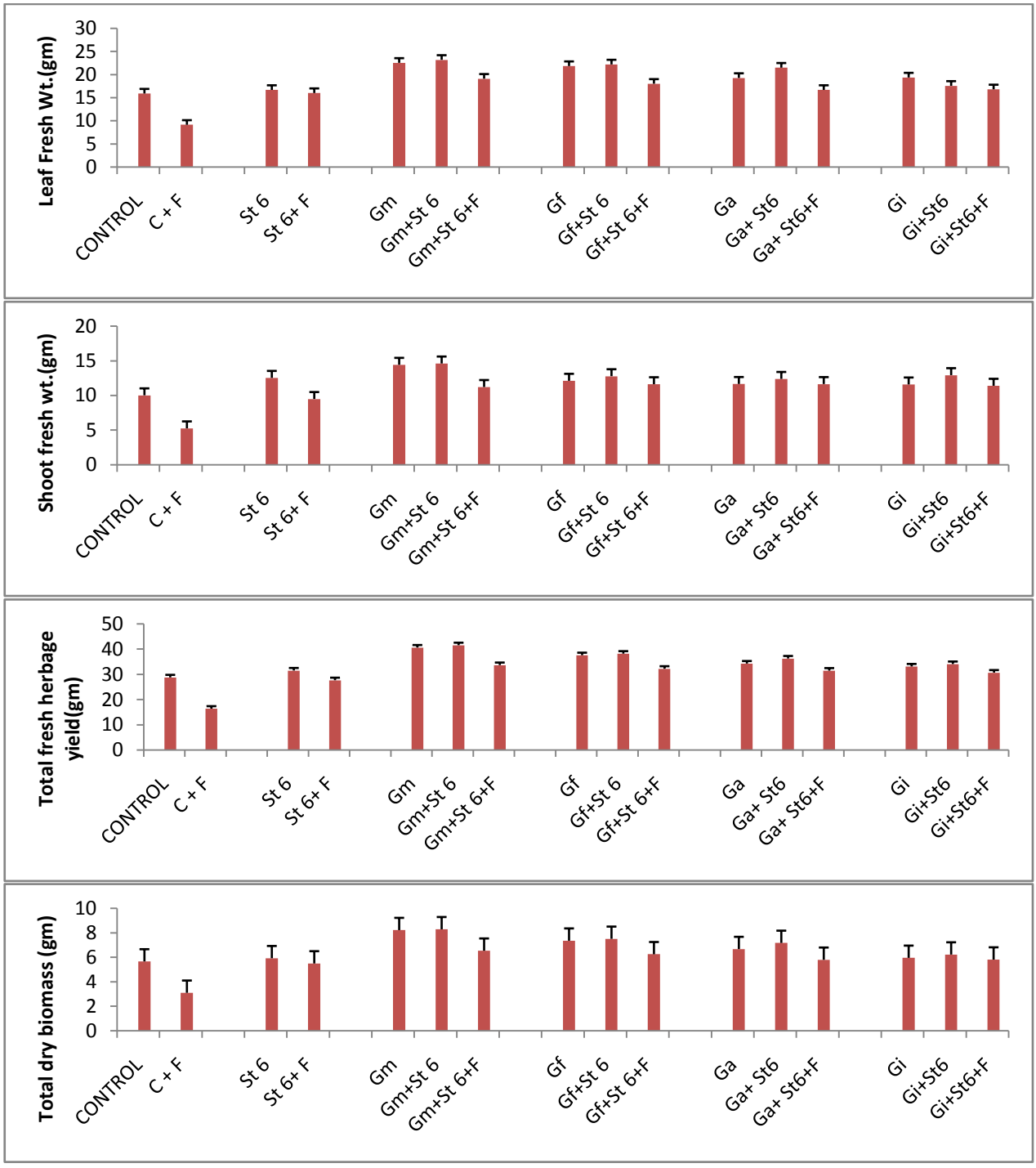
Spore Count and Percent Root Colonisation

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 $P \leq 0.05$.

3.Result



Graphs showing effect of various AM fungi and *B. lehensis*strain MLB2 on various plant parametersof *Ocimumsanctum.*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* var. CIM-AYU grown under NaF stress.

S.No	Treatments	Plant height (cm)	Shoot fresh weight(gm)	Leaf fresh weight (gm)	Total dry weight(gm)
1	CONTROL	85.913de	10.016e	16.246fg	5.663fg
2	C + F	57.700g	5.256 f	9.1433h	3.106h
3	St 6	82.840f	12.526bc	16.67ef	5.926ef
4	St 6 + F	82.460f	9.4855e	16.002g	5.500g
5	Gm	100.20ab	14.410a	22.533ab	8.220a
6	Gf	94.760ab	12.110bc	22.186ab	7.506b
7	Ga	92.766cd	11.636cd	21.486b	7.173bc
8	Gi	90.273bc	11.58cd	17.063c	6.22de
9	Gm+St 6	101.533a	14.60a	23.177a	8.386a
10	Gm+St 6+F	85.333de	11.21d	19.101cd	6.53d
11	Gf+St 6	95.250ab	12.773b	22.833ab	7.35b
12	Gf+St 6+F	83.706ef	11.616cd	18.006de	6.25de
13	Ga+St 6	93.726ab	11.650cd	19.263cd	6.67cd
14	Ga+ St 6+F	89.99cd	12.380bc	16.673ef	5.80ef
15	Gi+St6	90.897cd	12.923b	17.570ef	5.95ef
16	Gi+St 6+F	88.306cd	11.393cd	16.803ef	5.81ef

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

4.Discussion

Sodium fluoride has inhibitory effect on plant growth.Neutral to alkaline pH favors germination of *G. mossae*.Plants inoculated with AM fungi growing under saline conditions resulted in an increase in root length, fresh and dry weights of shoot and increased photosynthesis (Shhekoofeh and Sepideh 2011). Root length and shoot length, decreased in seedlings under fluoride stress (Gadi et al. 2012). 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.

5.Conclusion

Being a medicinally important plant, shoot of *Ocimum sanctum*is rich in essential oil.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 synergisticallycan prove to be a promissory note for better yielding of natural herbs in stress conditions.

6.Acknowledgement

Authors are sincerely thankful to the Head of Department of Botany, University of Lucknow, for providing the necessary laboratory facilities, Dr. Alok Kalra (senior Scientist, CSIR-CIMAP, Lucknow) for bacterial isolation & identification and CSIR-CIMAP, Lucknow for encouragement and facilities.

References

[1] Shetty S, Udupa S, Udupa L, 2008. Evaluation of antioxidant and wound healing effects of alcoholic and aqueous extract of *Ocimum sanctum* Linn in rats. *Evidence-Based Complementary and Alternative Medicine*; 5(1): 95-101

[2] Ghosh A, Bhardwaj M, Satyanarayan T, Khurana M, Mayilraj S, Jain RK. 2007. Bacillus lehensis sp. nov., an alkali tolerant bacterium isolated from soil. *Int J SystEvol Microbiology*; Feb 57(Pt 2):238-42

[3] Lakshmi V, 2013. Dental Fluorosis Prevalence among Children in Endemic Fluoride Areas of Chittoor District, *International journal of science and research*: Vol 2 Issue 12:109-111

[4] Dragland S, Senoo H, Wake K, Holte K, Blomhoff R. 2003. Several culinary and medicinal herbs are important sources of dietary anti- oxidants. *Journal of Nutrition* 133: 1286-1290

[5] Klimankova, E, Holadova, K, Hajˇslova, J, Cajka, T, Poustka, J, Koudela, M. 2008. Aroma profiles of five basil (*Ocimum basilicum* L.) cultivars grown under conventional and organic conditions. Food Chem. 107: 464–472

[6] Grayer R.G, Kite G.C, Goldstone F.J, Bryan S.E, Paton A, Putievsky E. 1996. Infraspecific taxonomy and essential oil chemotypes in basil, *Ocimum basilicum.Phytochemistry* 43, 1033–1039

[7] Smith SE and Read DJ. 1997. Mycorrhizal Symbiosis. 2nd edition. London: Academic press

[8] Aggarwal A., Kadian N, Tanwar A, Yadav A. and Gupta K.K. 2011. Role of arbuscular mycorrhizal fungi (AMF) in global sustainable development. *Journal of Applied andNatural Sciences*3(2): 340-351

[9] B.R. Gadi, VermaPooja and Amra Ram 2012. Influence of NaF on seed germination, membrane stability and some Biochemicals content in *Vigna*seedlings. *Journal of chemical, biological and physical sciences.* Vol.2. No. 3, 1371-137

[10] Shekoofeh E and Sepideh H. 2011. Effect of mycorrhizal fungi on some physiological characteristics of salt stressed *Ocimum basilicum* L. *Iranian Journal of Plant Physiology*, 1(4): 215-222

[11] McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. 1990. A new method which gives an objective measure of colonisation of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol*115:495-501

[12] Gerdemann JW & Nicolson TH. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society* 46:235–244

[13] Vyas D & Gupta RK. 2014. Effect of edaphic factors on the diversity of VAM fungi. *Tropical Plant Research* 1(1): 14–25