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Effects of various AMF and *Bacillus pumilus* strain NBRC 12092 on growth of *Ocimum sanctum* under salinity stress

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Abstract

The aim of the present study was to notice the synergistic effect of Arbuscular Mycorrhizal fungi (AMF) and *Bacillus pumilus*, on the growth of *Ocimum sanctum* var. CIM-AYU grown under 40 ppm of Sodium fluoride stress. *Glomus mossae* and *Bacillus pumilus* strain NBRC 12092 increased leaf fresh weight by 35% and 9.5% respectively. Consortium of *Glomus mossae* and *Bacillus pumilus* strain NBRC 12092 (Gm+St 2) resulted in 42% increase in leaf fresh weight of *Ocimum sanctum* as 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 pumilus* increased the fluoride tolerance level of the herb.

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

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) [6]. It is an annual herb native to India and other parts of Asia (Klimankova *et al*. 2008) [9]. The use of plant parts like root, stem and leaves has been maintained traditionally (Leonti *et al.* 2003). Medicinal herbs have been used from pre-historic times (Dragland *et al.* 2003) [3]. 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) [14].

Bacillus pumilus strain NBRC 12092 is a gram positive, aerobic, rod shaped and endospore forming bacteria (Ghosh *et al.* 2007) [5]. It shows high resistance to environmental stress. 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) [10]. Plant tolerance can be enhanced by AMF and their mutual relationship with plants help in their growth even in stressed conditions through improved physiological and nutritional stress (Hashem *et al*. 2015, 2016) [7, 8]. In this study, we studied the impact of fluoride stress on *Ocimum sanctum.* And also the ameliorating effects of various species of *Glomus* and *Bacillus pumilus* strain NBRC 12092 on *Ocimum sanctum.*

Material and Method

Sample collection

A bacterium, *Bacillus pumilus* strain NBRC 12092, 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 fluctuated 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 pumilus* 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 the physical parameters, spore count, % root colonisation and essential oil yield.

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) [4]. 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 ≤ 0.05.

Result

Table 1: Effects of various treatments involving different AMF and *Bacillus pumilus* strain NBRC 12092 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 plant dry biomass (gm)
1	CONTROL	68.333g	11.693ef	16.643ef	6.150f
2	C + F	52.446h	5.600h	7.033g	2.136h
3	St 2	90.203cd	12.923cd	18.236e	6.956f
4	St 2 + F	82.680f	10.126g	15.676 f	5.230g
5	Gm	94.760ab	14.410ab	22.533ab	8.220a
6	Gf	92.273bc	12.110de	22.186ab	7.506bc
7	Ga	96.880 ab	11.650ef	21.486bc	7.173cd
8	Gi	86.470ef	11.580ef	19.363d	6.226ef
9	Gm+St 2	100.200a	14.646a	23.680a	8.566a
10	Gm+St 2+F	93.726bc	12.060de	20.623cd	6.803de
11	Gf+St 2	96.366ab	14.270ab	22.883ab	8.210a
12	Gf+St 2+F	93.070bc	11.553ef	19.543d	6.476ef
13	Ga+St 2	89.903de	13.700ab	22.033bc	7.973ab
14	Ga+ St 2+F	96.700ab	11.090fg	19.130d	6.440ef
15	Gi+St 2	96.216ab	13.243bc	21.580bc	7.320cd
16	Gi+St 2+F	89.433de	12.386de	17.443e	6.100f

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

Where F: Sodium fluoride, St 2: *Bacillus pumilus* strain NBRC 12092, Gm: *Glomus mosseae*, Ga: *Glomus aggregatum*, Gf: *Glomus fasciculatum*, Gi: *Glomus intraradices*.

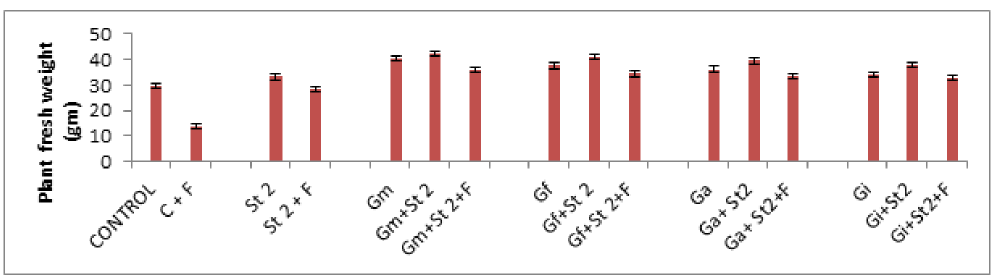

Fig 1

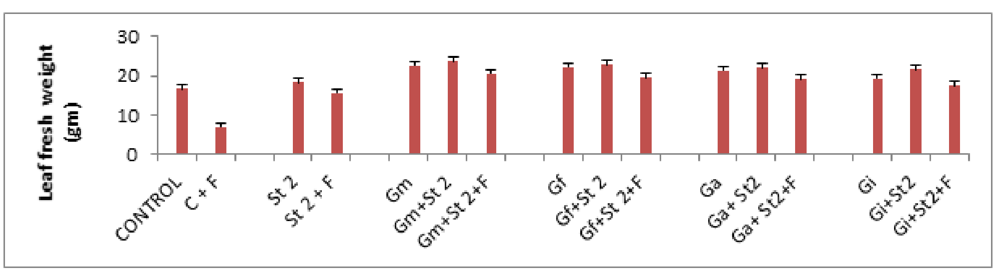

Fig 2

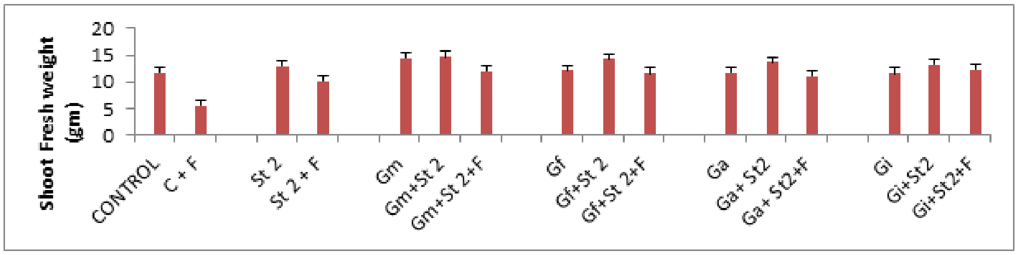

Fig 3

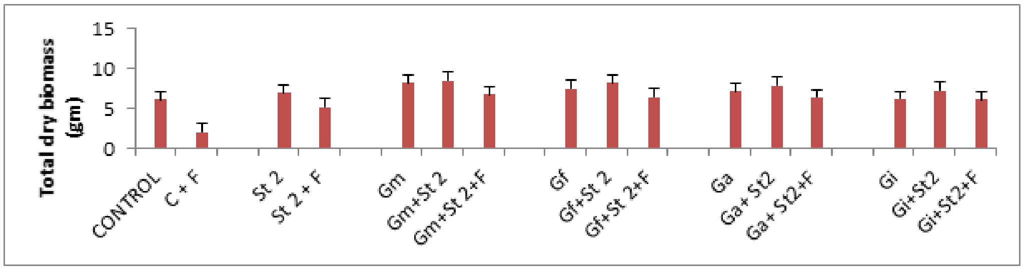

Figure: Histograms showing effect of various AM fungi and *Bacillus pumilus* strain NBRC 12092 on 1.) Plant fresh biomass, 2.) Leaf fresh weight, 3.) Shoot fresh biomass and 4.) Total plant dry biomass of *Ocimum sanctum.* Where F: Sodium fluoride, St 2: *Bacillus pumilus* strain NBRC 12092, Gm: *Glomus mosseae,* Ga: *Glomus aggregatum,* Gf: *Glomus fasciculatum,* Gi: *Glomus intraradices.*

Discussion

Sodium fluoride has an 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 *Bacillus pumilus* showed a remarkable coping effect of the herb against fluoride stress. *Glomus mossae* and *Bacillus pumilus* strain NBRC 12092 increased leaf fresh weight by 35% and 9.5% respectively. Consortium of *Glomus mossae* and *Bacillus pumilus* strain NBRC 12092 (Gm+St 2) resulted in 42% increase in leaf fresh weight of *Ocimum sanctum* as compared to control. Even in the presence of fluoride stress, the consortium showed a remarkable increase of 24% in leaf fresh weight. The best coping effect was shown by *G.mossae* followed by *G.fasiculatum*, *G.aggregatum* and *G. intraradices* respectively. Studies show that AM fungi are the root symbionts which improve plant health and mineral nutrition (Smith and Read 1997) [14]. So the results were coherent with the findings of earlier studies.

Conclusion

Being a medicinally important plant, shoot of *Ocimum sanctum* is rich in essential oil. Synergistic effect of AM fungi (AMF) inoculation with *Bacillus pumilus* 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 pumilus* used synergistically can prove to be a promissory note for better yielding of natural herbs in stress conditions.

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