Role of bioinoculants and auxin in development of salt tolerant *Mentha arvensis*

S. Kashyap, S. Sharma

Centre for Rural Development and Technology, Indian Institute of Technology, Hauz Khas, New Delhi, India

ABSTRACT: Pot experiments were conducted for the development of salt tolerant *Mentha arvensis* (Japanese mint, family: Labiatae) saplings involving bioinoculants, namely Arbuscular Mycorrhizal (AM) fungi, *Azotobacter* and an auxin – Indole Acetic Acid (IAA). The IAA and sodium chloride (NaCl) concentrations were standardized prior to the experiments. The 10-ppm IAA and 0.08% NaCl (w/v) were found to be optimum in combination with AM fungi and *Azotobacter* to increase all the growth parameters and microbial count in the rhizosphere. For development of salt tolerant saplings, the optimal concentration of IAA, along with AM fungi and *Azotobacter* in different combinations, was applied in pots. The saplings were irrigated regularly with 0.08% NaCl water. Although plant growth, AM infection percentage, AM spores/100 g soil and *Azotobacter* cells/g soil were affected by NaCl watering, the inoculation of both bioinoculants significantly enhanced survival percentage of saplings from 10 to 40% under salt stress. Maximum survival (40%) of saplings was found with IAA (10 ppm) + AM fungi + *Azotobacter* treatment.

Keywords: *Mentha arvensis*; *in vivo*; AM fungi; *Azotobacter*; IAA; NaCl stress

Mentha, an aromatic perennial herb (family Lamiaceae) is distributed mostly in the temperate and sub-temperate regions of the world. *Mentha arvensis* popularly known as Japanese mint or menthol mint is cultivated in India for its menthol rich essential oil used in medicine, cosmetics, food and flavour industry. It thrives well in sandy or loamy soils rich in humus. A well-drained soil with liberal irrigation is necessary (Kumar et al. 1999) and it cannot be grown on stressed especially salt affected soils. Further the polymorphic nature of Japanese mint associated with gynodioecy, polyploidy, and natural hybridization resulting in the production of male sterile/subfertile hybrids (Kukreja, Dhawan 2000) becomes a major problem in developing salt tolerant strains by plant breeding programs. Although extensive research work on various aspects of this species was conducted (Bhat et al. 2002; Maffei, Mucciarelli 2003) in the past few years, a systematic evaluation of varieties for saline areas is required.

Salinity of soil is one of the major environmental stresses limiting plant growth and productivity. There are 952 million ha of land under salinity and alkalinity in the world and out of this, 7 million ha of salt affected areas are in India (Yadav 2000). Agricultural practices like soil amelioration, irrigation facilities, agronomical managements, etc. may be energy intensive enterprise to modify the environment, but the economic impacts always limit the wide applicability of such practises. The development of tolerant plant genotypes by inducing salt tolerance through application of bioinoculants can be a practical solution to the problem of saline stress.

The bioinoculants like Arbuscular Mycorrhiza (AM) fungi and *Azotobacter* are reported to alleviate the saline stress and improve plant growth under a variety of salinity stress conditions (Ruiz-Lozano, Azcon 2000; Al-Karaki 2000) and further addition of suitable phytohormone concentrations (Kaldorf, Ludwig-Muller 2000) may increase the potential of these bioinoculants.

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Considering the above, pot experiments were conducted to evaluate the efficacy of native AM fungi and *Azotobacter* strains in combination with Indole Acetic Acid (IAA) for development of salt tolerant *M. arvensis* saplings under salinity stress.

MATERIALS AND METHODS

Standardization of IAA concentration

About 10–15 cm long suckers of *M. arvensis* with 8–10 leaves were collected during March and their basal ends were dipped in both low and high concentrations of IAA (5 ppm, 10 ppm, 15 ppm, 20 ppm, 1,000 ppm, 2,000 ppm, 3,000 ppm) solutions for about one hour. For each hormonal concentration, the following four treatments (with twenty replications each) were done in polythene bags (size 25 cm × 15 cm) containing 1.5 kg of soil mix (vermicompost:soil 1:3):

i) AM fungi (M),
ii) *Azotobacter* (A),
iii) AM fungi + *Azotobacter* (M + A),
iv) Control (C).

For treatment M, consortia of *Glomus mosseae, G. microcarpum, G. macrocarpum, G. fasciculatum, Gigaspora margarita* and *G. heterogama* were mixed (35g soil inoculum with about 75 AM spores/polythene bag). Treatment A was applied by dipping basal ends of hormone treated suckers in *Azotobacter* broth (with 10^7 to 10^9 bacteria/ml) for about half an hour before planting. For dual inoculation (M + A), both the treatments were done simultaneously and for controls (C) bags were kept with soil mix only.

One hormone treated sucker was planted in each bag and regular watering was done. Data pertaining to growth rate, i.e. shoot height, number of nodes/shoot and number of shoots/plant, survival percentage, fresh and dry weight and moisture percentage was collected after two months. AM infection percentage in roots, AM spore number/100 g of soil and *Azotobacter* cell count/g of soil were also monitored simultaneously by standard methods (Phillips, Hayman 1970; Gerdemann, Nicolson 1963; Subba Rao 1982). Based on the collected data, auxin concentration with the best plant growth and maximum AM and *Azotobacter* count was selected for further development of salt tolerant saplings.

Standardization of sodium chloride (NaCl) concentration

For this purpose, four hundred suckers (10–15 cm long) were planted individually in polythene bags (size 25 cm × 15 cm) with the same four treatments (M, A, M + A and C, 100 suckers/treatment) as used in the standardization of auxin.

The bags of each treatment were watered regularly with 0.01 to 0.10% NaCl (w/v) water, preventing leaching of salt from bags. Ten replications of each NaCl concentration were kept. The sprouting/survival % of suckers, pH and EC of bag soils were noted for one month (or 100% mortality of plants) at ten day intervals.

Development of salt tolerant saplings

For the development of salt tolerant saplings of *M. arvensis*, a standardized IAA concentration was applied with standardized doses of NaCl watering and native bioinoculants. Earthen pots (25 cm diameter × 30 cm depth) were filled with 4 kg soil mix with the same four treatments (M, A, M + A and C, 40 pots/treatment) as used in step 1 and 2. For treatment M, 100 g soil inocula (with 200 AM spores/100 g soil) were added into each pot and for treatment A, bacterial broth was applied to the suckers. The method of application was similar as used in the standardization of auxin.

Three hundred and twenty suckers (10–15 cm long with 8–10 leaves) were collected during March. Of these basal ends of one hundred and sixty were dipped in 10 ppm IAA (standardized concentration) for one hour and planted in a half of the pots (2 suckers/pot) filled with soil mix and applied different treatments. The rest of 160 suckers were planted without IAA in the other half of pots. Half of the pots with suckers treated with/without IAA were regularly watered with 0.08% NaCl (standardized concentration), while the other half was provided with regular tap watering (Tables 2 and 3). Precautions were taken to avoid the loss of solutions by leakage or overflow and pots were kept under natural conditions at the field site (temperature 28 ± 4°C, 12–14 hr. daylight).

Data pertaining to growth parameters, fresh and dry weights, moisture and survival percentage of saplings, pH, EC of pot soils, AM infection percentage in roots, AM spore count/100g soil and *Azotobacter* cell count/g soil was recorded for three months.

Statistical analysis

The data was analyzed by computer using the SPSS for Windows 9.0 package. Categorical data was compared using Chi-square analysis and Fisher's exact test when indicated (expected frequency of less than 5 in any cell). ANOVA was applied on quantitative variables with multiple groups followed by Scheffe's

Table 1. Standardization of IAA concentration*

IAA conc.	Treatments	Survival (%)	Growth parameters (average values)			*Azotobacter* cell count/g of soil	AM infection (%)	AM spore count/ 100g soil
			Shoot height (cm)	Number of nodes/shoot	Number of shoots/plant			
5 ppm	M	100^{a}	18 ± 2.1^{a}	12 ± 1.3^{a}	15 ± 2.4^{a}	1.8 × 10^{2}	45^{a}	50 ± 8.7^{ab}
	A	100^{c}	18 ± 2.6^{d}	14 ± 1.1^{d}	17 ± 1.4^{e}	5.7 × 10^{2}	28^{c}	32 ± 8.5^{ef}
	M + A	100^{e}	20 ± 3.0^{h}	18 ± 1.5^{g}	20 ± 2.0^{i}	6.8 × 10^{2}	52^{e}	55 ± 7.0^{h}
	C	90^{g}	15 ± 2.1^{l}	12 ± 2.4^{j}	15 ± 2.3^{m}	1.2 × 10^{2}	20^{g}	30 ± 4.6^{k}
10 ppm	M	100^{a}	22 ± 2.4^{a}	18 ± 1.4^{b}	20 ± 3.0^{b}	2.0 × 10^{2}	52^{a}	55 ± 5.8^{a}
	A	100^{c}	22 ± 3.0^{e}	18 ± 3.0^{e}	20 ± 2.2^{f}	6.3 × 10^{2}	30^{c}	35 ± 8.7^{e}
	M + A	100^{e}	25 ± 3.7^{i}	20 ± 1.5^{g}	25 ± 3.9^{j}	7.2 × 10^{2}	65^{e}	60 ± 7.3^{h}
	C	95^{g}	20 ± 1.9^{m}	15 ± 2.1^{k}	18 ± 2.7^{n}	1.5 × 10^{2}	25^{g}	32 ± 10.5^{k}
15 ppm	M	95^{a}	20 ± 2.7^{a}	12 ± 2.8^{a}	15 ± 2.1^{a}	1.5 × 10^{2}	42^{a}	50 ± 4.9^{ab}
	A	90^{c}	20 ± 2.4^{de}	12 ± 1.6^{d}	15 ± 2.1^{e}	5.8 × 10^{2}	25^{cd}	30 ± 9.6^{ef}
	M + A	95^{e}	22 ± 1.8^{hi}	15 ± 1.1^{h}	18 ± 2.2^{i}	6.0 × 10^{2}	50^{e}	52 ± 8.5^{hi}
	C	80^{g}	12 ± 2.4^{n}	12 ± 1.4^{j}	10 ± 1.7^{o}	1.2 × 10^{2}	20^{g}	28 ± 5.1^{k}
20 ppm	M	70	14 ± 1.4^{b}	8 ± 1.1^{c}	10 ± 2.3^{c}	1.2 × 10^{2}	40^{a}	43 ± 7.4^{ab}
	A	70	12 ± 2.4^{f}	8 ± 1.5^{f}	10 ± 1.8^{g}	5.0 × 10^{2}	20^{cd}	30 ± 9.8^{ef}
	M + A	75	15 ± 1.5^{j}	10 ± 0.9^{i}	12 ± 2.4^{k}	6.0 × 10^{2}	45^{e}	50 ± 5.3^{hi}
	C	65	10 ± 1.5^{no}	6 ± 1.6^{l}	8 ± 1.3^{o}	1.0 × 10^{2}	20^{g}	28 ± 6.5^{k}
1,000 ppm	M	60	10 ± 2.6^{c}	8 ± 1.3^{c}	6 ± 1.1^{d}	0.7 × 10^{2}	25^{b}	40 ± 6.3^{bd}
	A	60	10 ± 2.3^{fg}	7 ± 1.3^{f}	6 ± 1.6^{h}	3.0 × 10^{2}	20^{cd}	28 ± 7.1^{ef}
	M + A	70	12 ± 3.3^{jk}	10 ± 2.0^{i}	7 ± 1.4^{l}	3.2 × 10^{2}	25^{f}	42 ± 8.1^{ij}
	C	50	8 ± 1.7^{o}	6 ± 1.6^{l}	5 ± 1.7^{p}	0.5 × 10^{2}	20^{g}	25 ± 5.7^{k}
2,000 ppm	M	60	10 ± 2.6^{c}	7 ± 1.5^{c}	5 ± 1.3^{d}	0.6 × 10^{2}	22^{b}	30 ± 6.5^{cd}
	A	55	10 ± 2.6^{fg}	6 ± 1.6^{f}	5 ± 1.5^{h}	2.5 × 10^{2}	18^{cd}	20 ± 8.5^{fg}
	M + A	60	12 ± 1.8^{jk}	8 ± 1.5^{i}	6 ± 1.4^{l}	2.8 × 10^{2}	25^{f}	35 ± 7.5^{j}
	C	50	7 ± 1.5^{op}	5 ± 1.1^{l}	4 ± 1.1^{p}	0.4 × 10^{2}	18^{gh}	10 ± 4.9^{l}
3,000 ppm	M	50^{b}	8 ± 2.1^{c}	7 ±.1^{c}	4 ± 1.1^{d}	0.6 × 10^{2}	20^{b}	25 ± 12.4^{d}
	A	50^{d}	8 ± 1.4^{g}	6 ± 1.3^{f}	4 ± 1.3^{h}	2.0 × 10^{2}	15^{d}	15 ± 6.4^{g}
	M + A	55^{f}	10 ± 1.4^{k}	8 ± 1.1^{i}	5 ± 1.6^{l}	2.0 × 10^{2}	20^{f}	30 ± 9.3^{j}
	C	40^{h}	5 ± 1.1^{p}	4 ± 1.1^{l}	3 ± 1.1^{p}	0.4 × 10^{2}	15^{h}	10 ± 5.4^{l}

*2 months data, M = AM fungi, A = *Azotobacter,* C = control
Mean/percentage followed by the same letter/without any letter is not significant at p = 0.05 level. All comparisons were done between similar treatments (M, A, M + A, C) at different concentrations of IAA

multiple comparison test. Quantitative variables with normal distribution and equal variance were compared by two-tailed t-test. The Mann-Whitney U nonparametric test was used for non-normal data.

RESULTS

Standardization and selection of IAA concentration

All the growth parameters, survival percentage and microbial count were found to be the best on M+A treatment with 10 ppm of IAA to suckers. The 100% survival was noted at 5 and 10 ppm of IAA with M, A and M + A treatments, while in controls it was 90% (at 5 ppm) and 95% (10 ppm). At higher concentrations of auxin (3,000 ppm), survival rate as well as plant growth were reduced significantly (Table 1).

Overall, the effect of hormonal treatments on growth was in the order 10 ppm > 5 ppm > 15 ppm > 20 ppm > 1,000 ppm > 2,000 ppm > 3,000 ppm. Depending upon the results, 10 ppm was found to be an optimum concentration for increasing the potential of AM fungi and *Azotobacter* and was

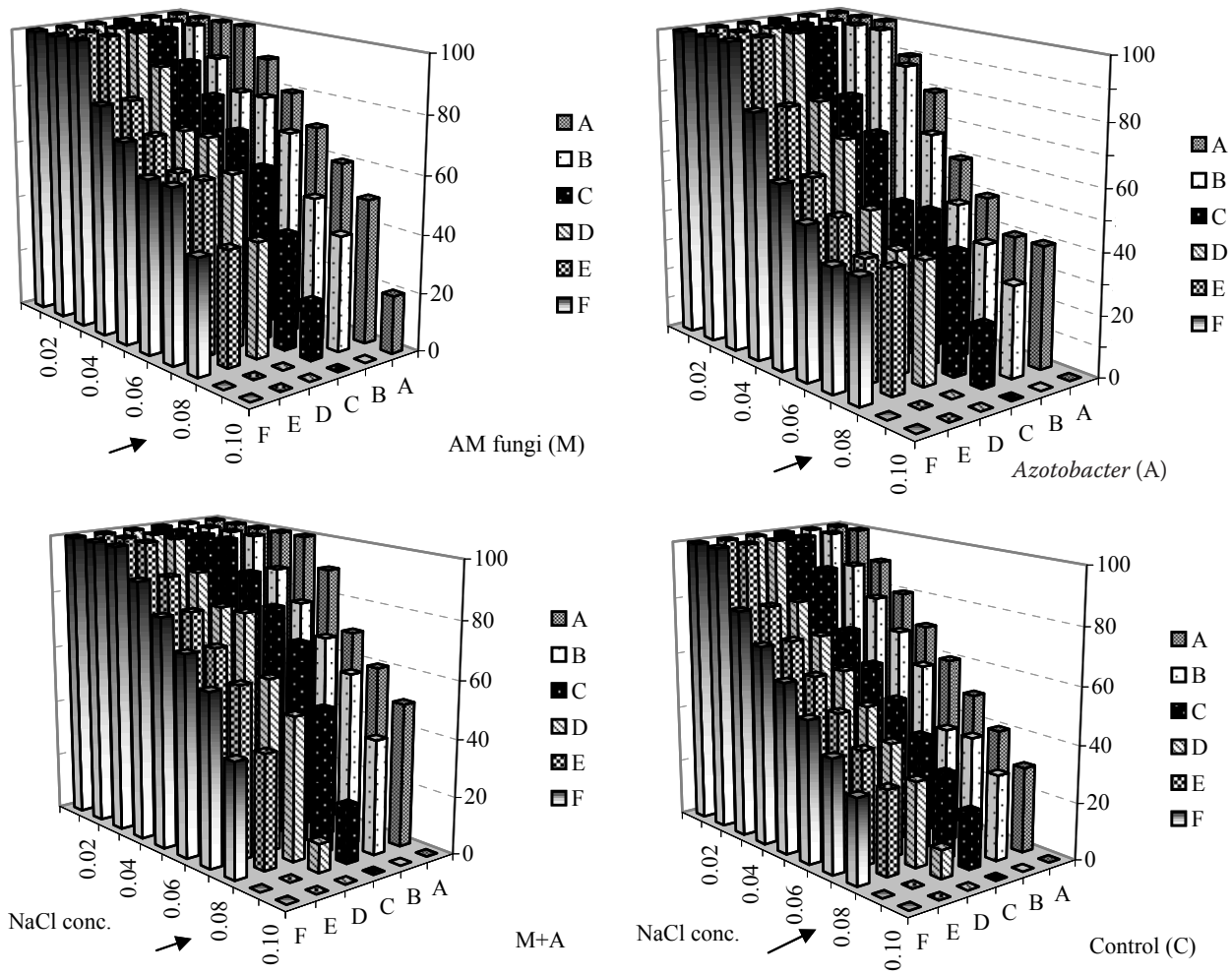

Fig. 1. Effect of NaCl watering on sprouting/survival percentage of *Mentha arvensis* in different treatments
→ = selected NaCl concentration
A = after 10 days, B = after 20 days, C = after 30 days, D = after 40 days, E = after 50 days, F = after 60 days

utilized further for the development of salt tolerant saplings.

Standardization and selection of NaCl concentration

It was noted that with low NaCl concentration (0.01 to 0.02%) watering, 100% plants survived for 2 months in all cases (i.e. M, A, M + A and C) and by increasing salt stress, survival percentage was reduced drastically. It was reduced to 40% in M, A and M + A and to 30% in C at 0.08% NaCl in 60 days (Fig. 1). EC was increased to 4.743 mmhos/cm in M, 4.690 mmhos/cm in M + A, 4.845 mmhos/cm in A and 4.870 mmhos/cm in C (Fig. 2). But no variation was noted in pH with saline watering among all the treatments (Fig. 3). Higher NaCl concentration (> 0.08%) resulted in 100% mortality of plants after 60 days.

Depending upon survivability of plants for 2 months with different bioinoculant treatments, 0.08% NaCl watering was selected for further development of salt tolerant saplings.

Development of salt tolerant saplings

Effect of different treatments on plant growth and survival

The results related to development of salt tolerant saplings are summarized in Tables 2 and 3. Maximum survival percentage was observed in M + A + 10 ppm IAA (40%) followed by M + H, A + H, M + A (35%), M, A (30%), H (15%) and C (10%) under salt stress while 100% plants survived at M + A + H, A + H and M + H treatments without NaCl watering (Fig. 4).

The plant growth with respect to shoot height, number of shoots and node number decreased significantly with 0.08% NaCl watering as compared to watering without NaCl (Table 2). The inclusion of AM fungi and *Azotobacter* with hormonal treatments significantly increased the growth rate and

Table 2. Effect of different treatments on growth of *Mentha arvensis* saplings**

Treatments	Growth parameters (average values)						Average fresh weight/plant (g)		Average dry weight/plant (g)		Moisture (%)/plant	
	Shoot height (cm)		Number of nodes/shoot		Number of shoots/plant							
	–S	+S	–S	+S	–S	+S	–S	+S	–S	+S	–S	+S
M	24 ± 2.9^{ad}	22 ± 2.6^{ac}	20 ± 3.3^{ab}	16 ± 2.1^{abc}	20 ± 2.4^{abc}	10 ± 2.3^{ad}	28.5 ± 2.1^{ac}	20.5 ± 2.5	7.9 ± 0.4^{a}	5.6 ± 0.7^{ac}	72.2	72.68
M + H	28 ± 2.1^{ab}	24 ± 3.1^{a}	22 ± 2.6^{a}	18 ± 3.0^{ac}	22 ± 2.4^{ab}	14 ± 1.5^{b}	30.2 ± 1.2^{a}	20.9 ± 4.1	8.6 ± 0.5^{a}	5.8 ± 0.5^{ac}	71.52	72.24
A	24 ± 2.5^{ad}	20 ± 3.3	16 ± 1.9^{bc}	14 ± 1.5^{b}	18 ± 2.0^{ad}	10 ± 1.3^{ad}	30.0 ± 4.2^{a}	20.0 ± 2.3	8.2 ± 0.3^{a}	5.4 ± 0.3	72.66	73.0
A + H	25 ± 3.7^{abd}	22 ± 3.4^{ac}	20 ± 2.8^{ab}	16 ± 2.2^{abc}	22 ± 3.1^{ab}	12 ± 2.4^{ab}	30.2 ± 0.9^{a}	20.8 ± 0.5	8.4 ± 0.5^{a}	5.8 ± 0.5^{ac}	72.18	72.1
M + A	27 ± 3.4^{ab}	22 ± 2.4^{ac}	20 ± 3.7^{ab}	18 ± 2.5^{c}	25 ± 3.9^{bc}	12 ± 2.2^{ab}	28.5 ± 1.9^{ac}	21.5 ± 1.0^{a}	7.9 ± 0.4^{a}	6.0 ± 0.5^{a}	72.28	72.09
M + A + H	30 ± 3.6^{b}	25 ± 3.9^{a}	22 ± 3.1^{a}	20 ± 2.4^{c}	28 ± 3.1^{c}	15 ± 2.3^{b}	30.5 ± 1.0^{a}	22.7 ± 1.7^{a}	8.6 ± 0.4^{a}	6.2 ± 0.5^{a}	72.13	72.68
C	18 ± 2.0^{c*}	16 ± 2.0^{b}	12 ± 2.4^{c}	10 ± 2.4^{d}	15 ± 3.6^{d}	5 ± 1.1^{cd}	20.2 ± 2.4^{b}	17.0 ± 2.0^{b}	5.6 ± 0.5^{b}	4.6 ± 0.5^{b}	72.76	72.72
H	20 ± 2.4^{d*}	18 ± 2.3^{bc}	15 ± 1.8^{c}	10 ± 1.6^{d}	16 ± 2.4^{d}	7 ± 1.1^{d}	25.7 ± 2.9^{c}	18.7 ± 3.1	7.0 ± 0.5^{c}	5.1 ± 0.5^{c}	72.51	72.10

**3 months data, M = AM fungi, A = *Azotobacter*, C = control, H = IAA (10 ppm)
–S – saplings developed with normal watering
+S – saplings developed with saline (0.08%) watering
*no significant difference between two treatments ($p > 0.05$)
Mean/percentage followed by the same letter/without any letter is not significant at $p = 0.05$ level. All comparisons were done between different treatments

Table 3. Effect of different treatments on microbial count in *Mentha arvensis* rhizosphere*

Treatments	*Azotobacter* cell count/g soil		AM infection (%)		AM spore count/100 g soil		EC of pot soil		pH of pot soil	
	+S	+S	–S	+S	–S	+S	–S	+S	–S	+S
M	2.6×10^2	0.4×10^2	58^{a}	48^{a}	60 ± 6.9^{a}	35 ± 5.0^{ab}	0.110	5.035	7.50	7.56
M + H	2.8×10^2	0.6×10^2	62^{a}	50^{a}	65 ± 4.9^{ac}	38 ± 5.1^{ab}	0.110	5.022	7.51	7.53
A	6.2×10^2	0.8×10^2	35^{b}	30^{b}	42 ± 4.7^{b}	30 ± 4.8^{a}	0.112	5.032	7.52	7.52
A + H	6.5×10^2	0.9×10^2	40^{b}	32^{b}	45 ± 5.8^{b}	32 ± 4.8^{ad}	0.114	5.035	7.51	7.50
M + A	7.0×10^2	1.0×10^2	56^{a}	52^{a}	60 ± 5.3^{a}	38 ± 4.0^{ab}	0.110	5.022	7.50	7.54
M + A + H	7.5×10^2	1.2×10^2	70^{a}	55^{a}	70 ± 6.7^{c}	40 ± 4.5^{b}	0.110	5.015	7.53	7.52
C	1.5×10^2	0.2×10^2	32^{b}	25^{b}	40 ± 4.6^{b}	22 ± 4.0^{cd}	0.112	5.090	7.50	7.53
H	1.8×10^2	0.3×10^2	35^{b}	30^{b}	42 ± 4.5^{b}	25 ± 4.4^{d}	0.112	5.065	7.50	7.54

*3 months data, M = AM fungi, A = *Azotobacter*, C = control, H = IAA (10 ppm)
–S – saplings developed with normal watering
+S – saplings developed with 0.08% NaCl watering
Mean/percentage followed by the same letter/without any letter is not significant at $p = 0.05$ level. All comparisons were done between different treatments

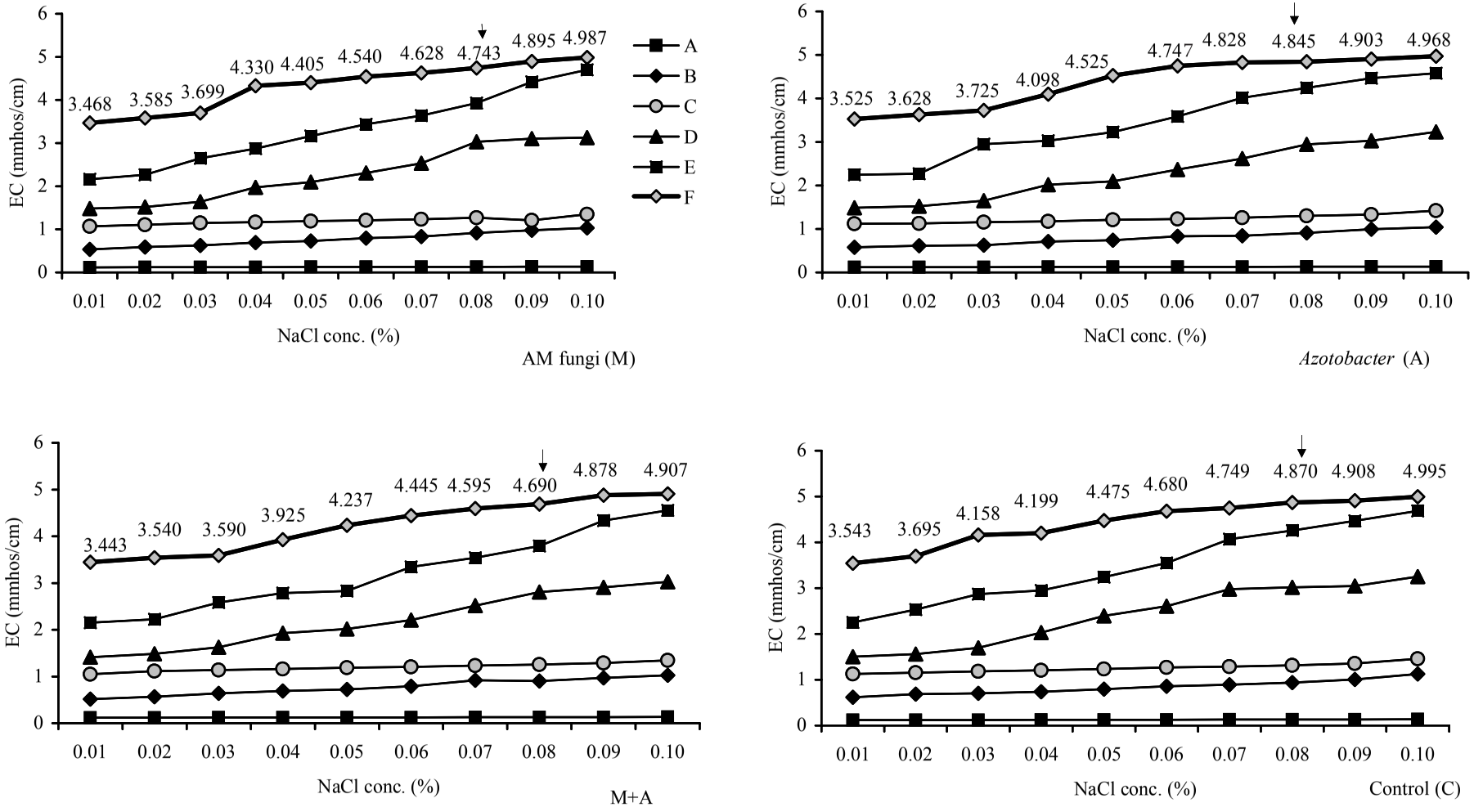

Fig. 2. Effect of NaCl watering on EC of soil in *Mentha arvensis* in different treatments
→ = EC at selected NaCl concentration
A = after 10 days, B = after 20 days, C = after 30 days, D = after 40 days, E = after 50 days, F = after 60 days

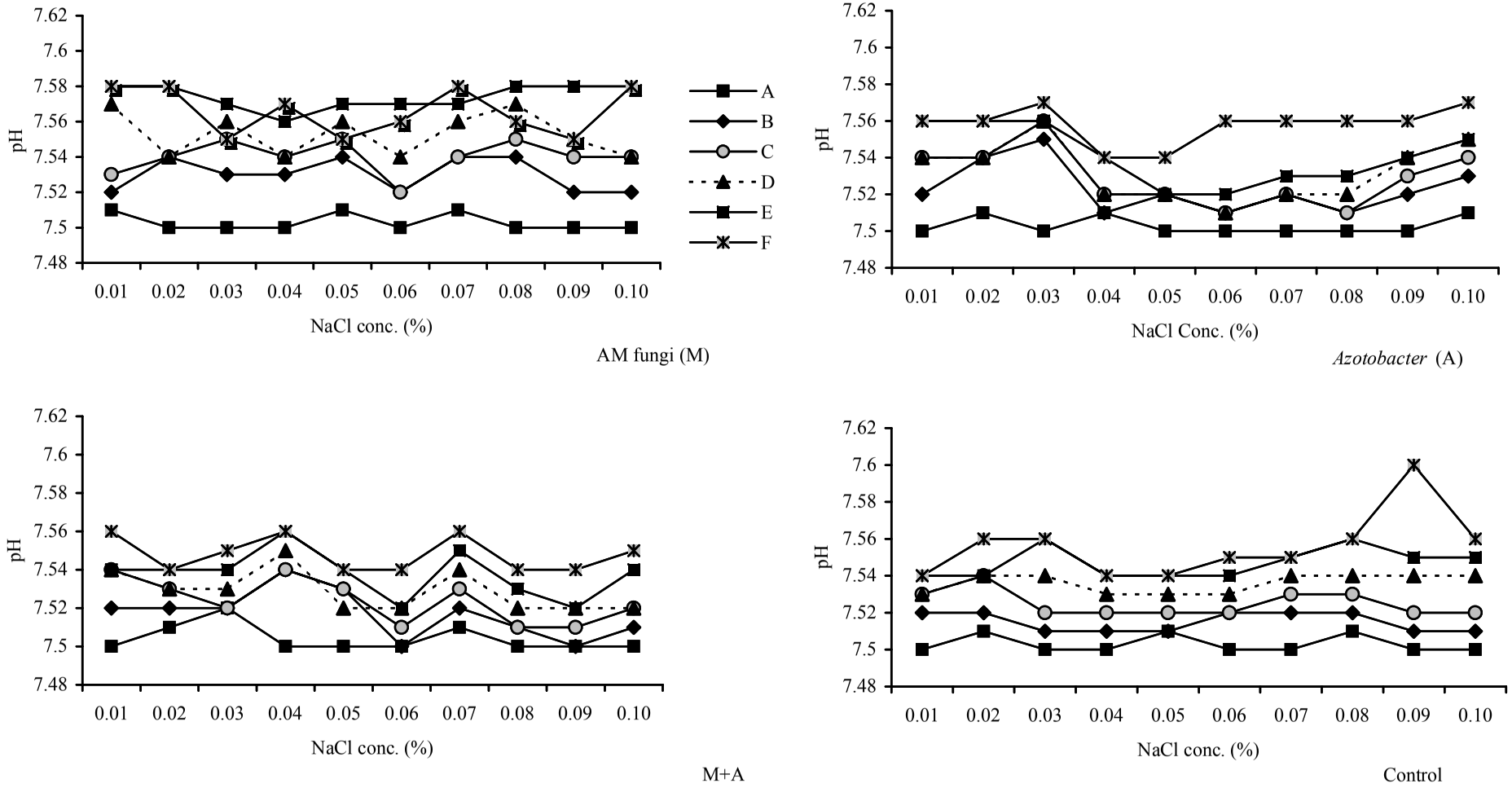

Fig. 3. Effect of NaCl watering on pH of soil in *Mentha arvensis* in different treatments
A = after 10 days, B = after 20 days, C = after 30 days, D = after 40 days, E = after 50 days, F = after 60 days

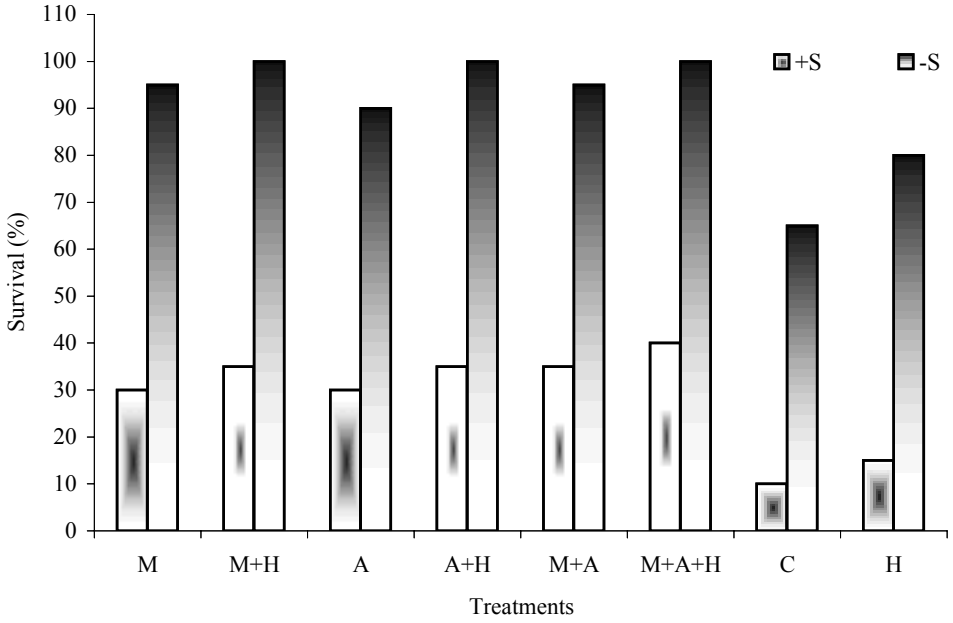

Fig. 4. Survival percentage of salt tolerant saplings of *Mentha arvensis*
M = AM fungi, A = *Azotobacter*, C = control, H = IAA (10 ppm)
+S = saplings developed with saline watering
–S = saplings developed with normal watering

fresh and dry weight of saplings ($p < 0.05$) against the control. A significant difference was also observed between –S and +S treatments with respect to all the observed parameters except moisture % of plants.

Effect of different treatments on microbial number in plant rhizosphere

The microbial number was found to be decreased significantly by salty water treatment. The maximum AM spore count and AM infection % were observed in M + A + H and M + H (–S) treatments, while under salt (+S) stress, M, M + H, M + A and M + A + H significantly increased these parameters against the control. The *Azotobacter* number/g soil was reduced from 7.5×10^2 to 1.2×10^2, AM spore count/100 g soil from 70 to 40 and AM infection percentage from 70 to 55% in M + A + H by 0.08% NaCl watering. The EC of pot soils after 3 months ranged between 5.015 and 5.090 mmhos/cm in different bioinoculant treatments with NaCl watering but not many variations were observed in pH (Table 3).

DISCUSSION

Standardization and selection of IAA concentration

The high concentrations of auxins were not found favourable in the present study. The results are consistent with the previous observations that auxins generally promote rooting at lower concentration and inhibit it at higher concentration, and that the optimal concentration for rooting varied with the plant species and the nature of the auxins (Gryndler et al. 1998). The high proportion of growth regulators could produce karyotypic alterations and physiological (and sometimes genetic) effects (McHughen, Swartz 1984) resulting in growth retardation.

The external application of phytohormones to the rhizosphere also stimulates mycorrhizal formation and mycorrhizal fungi themselves produce several growth promoting hormonal substances (Kaldorf, Ludwig-Muller 2000) and may alter the plant internal hormone balance. An increase in AM colonization by a mixture of GA_3, cytokinins and auxins has been documented (Azcon et al. 1978). The mycorrhiza has been found to have a strong synergistic effect with auxins modifying the nutritional and hormonal pattern of fruit rootstock cuttings and resulting in enhancement of rooting (Cristoferi et al. 1985). Similarly, the secretion of several hormones by *Azotobacter* (Subba Rao 1982) and enhancement of bacterial number and nitrogen fixing ability of *Azotobacter* by external application of hormones was reported (Kukreja et al. 1995). The cell number of *A. chroococcum* increased from 10^5 to 10^8 within eight days of incubation by added hormones and maximum growth was observed with IAA and GA_3

in their studies (Kukreja et al. 1995). In our study, 10 ppm IAA concentration was found best to act synergistically with AM fungi and *Azotobacter* in improving all the parameters. The inhibitory effect of auxins at higher concentrations could be the result of supra optimal endogenous level of auxins induced by the treatment of AM fungi and *Azotobacter* (Stein, Fortin 1990).

Standardization and selection of NaCl concentration

The survival and growth of bioinoculated plants with low concentration NaCl watering in the present study are supported by Stein and Fortin (1990). The high NaCl concentration might have affected AM fungi and *Azotobacter* (Villar-Arteaga, Zuniga-Davila 1997) and resulted in decreased survival percentage. The adverse effects of high salt concentration on mycorrhizal propagules in soil, AM root infection and plant growth, which could be due to the inhibition of hyphal growth because of reduction in supply of carbohydrates from the plant to the fungus, were reported (McMillen et al. 1998).

Development of salt tolerant saplings

Effect of different treatments on plant growth and survival

The present results related to the potential of AM fungi in enhancing plant growth under stress conditions are supported by Hirrel and Gerdemann (1980) and Selvaraj and Manivannan (1997). The AM fungi seem to avoid NaCl stress through an ecophysiological mechanism (Stein, Fortin 1990). The composite root-fungus surface area may provide a larger contact of plant with soil. Moreover, the fungus hyphae may provide a low resistance pathway for water to flow from the rhizosphere to the root stele (Scagel, Linderman 1998).

AM fungi are also known to influence the composition of amino acids and carbohydrates of the host plant. The increased salt tolerance by AM fungi is also probably due to improved P nutrition (Jindal et al. 1995). The increased level of plant growth hormones in mycorrhizal association (Kaldorf, Ludwig-Muller 2000) may additionally help plants under stress conditions as shown in the present study. The best results in the combination of M + A + H could be due to their combined positive effects exerted towards the growth of plants.

The synergistic effect of *Azotobacter* and mycorrhiza might have played a role in increasing the survival percentage of *M. arvensis* under NaCl stress in the present study. In the dual inoculation, nitrogen fixers increase the incidence and population of AM fungi as AM fungi have a special requirement for nitrogen resulting in better N and P uptake, secretion of phospho-enzyme and other growth promoting substances (Barea et al. 1997). Similarly, the root exudates from AM plants contain high amounts of amino acids, organic acids and sugars which are important for an enhanced chemotactic response of *A. chroococcum* to AM fungi (Gupta Sood 2003).

Effect of different treatments on microbial number in plant rhizosphere

The decrease in microbial count in the present study may be due to osmotic or toxic effects related to increasing NaCl concentrations (Juniper, Abbott 1993). The AM hyphal growth in saline soil is dependent on the maintenance of ionic balance and internal water potential in the mycelium to maintain turgor and these processes require energy. It is therefore possible that the capacity for hyphal growth was reduced more rapidly in soil with NaCl due to an increased energy requirement. However, treatments with AM fungi alone and in combination with auxin and *Azotobacter* significantly revealed a higher AM infection percentage ($p < 0.05$) as compared to others (Table 3).

In the present study, overall salinity affected plant survival (%), growth and microbial associations in the rhizosphere as compared to normal soil conditions but inoculation of bioinoculants, specifically AM fungi, played a significant role. Plants colonized by AM fungi have a higher percentage of photoassimilates and exhibit higher root/shoot ratios than non-mycorrhizal ones (Clapperton, Reid 1992). The content of soluble sugars is found to be increased in the roots or leaves of mycorrhizal plants, which could support osmotic adjustment in order to compensate for decreased soil water potential (Nelson, Achar 2001). In the present study, the positive effect of AM fungi under salt stress situations could be due to these facts. Further the addition of auxins may increase the activity of ATPases at the plant-fungus interface (Gogala 1991) and assist mycorrhiza to enter the host plant and promote the arbuscule formation process. In the present study, the optimum level of auxins may have enhanced the ATPase activity to some extent and subsequently increased the activity of bioinoculants (Smith, Smith 1990), which further promoted the plant growth and survival under salt stress.

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Úloha bioinokulantů a auxinu při vývoji máty *Mentha arvensis* tolerantní vůči soli

ABSTRAKT: Uskutečnili jsme nádobové pokusy zaměřené na vývoj sazenic máty *Mentha arvensis* (máta rolní, čeleď: Labiatae) tolerantních vůči soli, kde jsme jako bioinokulanty použili arbuskulární mykorhizní houby (AM), azotobakter a auxin – kyselinu indolyloctovou (IAA). Před začátkem pokusů jsme provedli standardizaci koncentrací IAA a chloridu sodného (NaCl). Z hlediska zvýšení všech růstových parametrů a mikrobiálního počtu v rizosféře jsme jako optimální shledali 10 ppm IAA a 0,08 % NaCl (váhového objemu) v kombinaci s houbami AM a azotobakterem. Pro vývoj sazenic tolerantních vůči soli jsme aplikovali optimální koncentraci IAA spolu s houbami AM a azotobakterem v různých kombinacích. Sazenice jsme pravidelně zalévali vodou s 0,08 % NaCl. Ačkoliv zálivka vodou s NaCl ovlivnila růst rostlin, procentuální infekci AM, počet spor AM na 100 g zeminy a počet tyčinek azotobakteru na 1 g půdy, použití obou inokulantů významně zvýšilo při solné zátěži procento přežití sazenic z 10 na 40 %. Maximální přežití (40 %) sazenic jsme zaznamenali ve variantě IAA (10 ppm) + houby AM + azotobakter.

Klíčová slova: *Mentha arvensis*; *in vivo*; houby AM; azotobakter; IAA; solná zátěž

Corresponding author:

Dr. Suman Kashyap, Centre for Rural Development and Technology, Indian Institute of Technology, Hauz Khas, New Delhi – 110016, India
tel.: + 91 11 265 962 29 (Off), + 91 11 263 033 61 (Res), fax: + 91 11 265 911 21, e-mail: skkashyap@hotmail.com

INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION
Slezská 7, 120 56 Prague 2, Czech Republic
Tel.: + 420 227 010 111, Fax: + 420 227 010 116, E-mail: redakce@uzpi.cz

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