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# Rhizobacteria and zeolites for overcoming saline stress in the cultivation of succulentplants

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--------------------------------------------------***ABSTRACT***------------------------------------------------------
*The aim of the researchwas to improve the growth and quality of plants of Euphorbiamilii and Crassula ovata, usingsubstrates with addedzeolites and Effectivemicroorganisms and assesswhethertherewas the possibility of alleviate stress caused by the presence of NaCl.*
*The experiment on Euphorbia and Crassula plantinvolvedthreetheses: 1) soil; 2) soil with addition of chabazitic-zeolites; 3) soil with addition of chabazitic-zeolites and treated with EM microorganisms.*
*The results showed that the use of zeolites and EM microorganisms increase the quality characteristics of Euphorbia milii and Crassulaovata under cultivation, in terms of plant growth, root development and mitigation of the stress caused by the presence of NaCl in the substrate.*

***KEYWORDS:*** *substrates, euphorbia,crassula, plant quality, EM, chabazite, abiotic stress*
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## I. INTRODUCTION

Plants are linked to theirenvironment, wheretheyusually live naturallyprotected, and are ableto complete their life cycle. However, duringtheir life, they are exposed to extensivechanges in environmentalconditions and numerousstressorsthat, depending on theirduration and intensity, reduce the vitality of the plantscausingdamage. Plantgrowth and developmentdepend on the interactions of the genotype (information contained in the genetic code of the plantat the level of the chromosomes) with variousexternalfactors, suchas: light intensity, temperature, water and nutrientavailability and salinity. [2]Every time thereis a variation in thesefactors in the environment, either in defect or in excess, wespeak of "stress" because the living organismissubject to potentiallyharmfulchanges. [2] Sometimes the plantsorganizethemselvesinternally so as to prevent the cells from beingsubjected to stress avoiding the unfavourableconditions: avoidance of stress; othertimestheysurvivenormallytolerating the unfavourableconditions: tolerance to stress. In the naturalenvironment, plants are able to quicklyacclimatise and adapt to specificconditions, developingparticularmechanisms of tolerance to stress. However, they are oftenexposed to sudden short- or long-term stress phenomenathat reduce cellularactivity by minimizingplantgrowth and eventuallyleading to death in the mostseriouscases. Especially in pottedcropsthisaspectplays an importantrole, asplantssubject to saline substratespresentbothosmotic stress and toxicityphenomenathatgenerally cause a weakenedrootsystem, lownutrientabsorptioncapacity and lowphotosyntheticactivity. Allthisresults in a significantloss of production. In thisarticle are presented the results of plantsf *Euphorbiamilii* and *Crassula ovata*, cultivated in alternative substratesbased on zeolites and microorganismsEm, to whichwasadministeredmonthlysodiumchloride. Thisis to assesswhetherzeolites and microorganismsmayhaveanyinfluence on the resistance of plants to saline stress.
EM includes a selection of live cultures of naturallyisolatedsoil micro-organisms [8][18][19]; EM micro-organisms include 83 bacterial and fungalstrains of differentspecies[9], of optional aerobic and anaerobictypes. Theirmainactivityis to increasesoilbiodiversity and stimulateplantgrowth[3].

Zeolites are particulardiagenizedpyroclasticrocks with peculiarproperties (high and selectivecationexchangecapacity,high water retention, micro- and macro-textileporosity) thatjustifytheiradvantageousapplications in the agronomic and floricfield [13]; in particular, chabazitic-zeolites(a type of which the Italiansoilisveryrich) can be used in substrates to replaceinertmatrices (vermiculite, perlite, pumice) with significanteffects on plantdevelopment and resistance to stress, reducing the use of water and fertilizers[10] [1][11].

## II. MATERIAL AND METHODS

**Greenhouse experiment and growing conditions**

The experiment, whichbegan in earlyOctober 2016, wascarried out at a localgrower in Rosignano Solvay (LI) on plants of *Euphorbiamilii* and *Crassula ovata*. The experimentinvolvedrootedcuttings of 3cm for *Euphorbiamilii*and 4cm for *Crassula ovata* , placed in pots ø 10 cm, in threedifferentmixtures of substrates to assesstheirgrowth and flowering. 50 plantswereused for 3 replicas, for 3 theses, 450 plants in total for eachcultivatedspecies, in a completelyrandomizedexperimentalscheme. To thesewasadded 50 mM of NaCl once every 15 days.

The 3 experimentaltheses in cultivationwere:

- Control (CTRL): soil for acidophilic 40%, volcaniclapillus 30%, quartzsand 30%, trivalentfertilizer 7-14-21 (pH 6);
- Treated (T1): acidophilicsoil 40%, chabazitic-zeolites 20%, quartzsand 40%, trivalentfertilizer 7-14-21 (pH 6);
- Treated (T2): soil for acidophilic 40%, chabazitic-zeolites 20%, quartzsand 40%, microorganisms EM dilution 1:100 (rootwetting and treatment once a month), trivalentfertilizer 7-14-21 (pH 6);

For eachspecies under cultivationwasevaluated: heightplant, leavesnumber and flowersnumber (for Euphorbia) and the totalplantfreshweight. In addition, the rootfreshweightwasmeasured.

The analysis of zeolitesused in the testshad a zeolithiccontentdetermined by X-raysusing the Rietveld-RIR methodology[5]: 67 ± 3% of which 64% given by chabazite and 3% by phillipsite. The cationexchangecapacity (CSC) determined by exchange with 1 N solution of $NH_4$according to the methodologydescribed in Gualtieri et al. [4]: 210 ± 10 meq/100g, of which 131 are due to Ca, 68 to K, 7 to Na and 4 to Mg.

**Statistics**

The experimentwascarried out in a randomized complete block design. Collected data wereanalysed by one-way ANOVA, using GLM univariate procedure, to assesssignificant ($P \leq 0.05$, 0.01 and 0.001) differencesamongtreatments. Meanvalueswerethenseparated by LSD multiple-range test ($P = 0.05$). Statistics and graphicsweresupported by the programsCostat (version 6.451) and Excel (Office 2010).

## III. RESULTS

The test showedthat EM microorganisms and chabazitic-zeolites can improve the quality and cultivationcharacteristics of slow growingspeciessuchasEuphorbia and *Crassula ovata*, despite the monthlyadministration of NaCl. In (Tab.1) on *Euphorbiamilii*, itisnotedthat the mixturescontaining 20% chabazitic-zeolites and 20% EM plus chabazitic-zeolites, significantlyincreased the plantsheight (16.13 and 23.50 cm against 10.22 cm of the control), the leavesnumber (18.22 and 24.06 against 14.02 of the control), the flowersnumber (20.14 and 31.08 against 8.11 of the control) (Fig.2) and the totalfreshweight of the plant (24.33 and 32.55 against 18.60 of the fertilized control). Also in *Crassula ovata* (Tab.2) in the theseschabazitic-zeolites 20% and chabazitic-zeolites 20% plusEffectivemicroorganisms, thereis a significantincrease in the plantsheight, the leavesnumber and the totalfreshplantsweight. In particular 13.21 and 19.53 cm respectively, against 8.13 cm of the control for the plantsheight, 22.33 and 28.05 against 16.13 of the control for the leavesnumber and 35.31 and 41.50 against 29.44 for the totalfreshplants.weight.

Fig. 1 shows how the differenttypes of substrate can influencerootdevelopment, in particularchabazitic-zeolites and the microorganismsEmseem to reduce the stress caused by the addition of NaCl in bothEuphorbia and Crassula.

## IV. DISCUSSION

The use of zeolites and EM microorganisms can guarantee, asdemonstrated by thisevidence, a clearimprovement in the quality of the plants in cultivation in terms of plant and rootgrowth and in the number of flowers. Aspectsalsohighlighted on otherornamental and horticulturalspecies [15][17][16][10][1][12][14][6][7]. The treatment with microorganisms and zeolites in the cultivationsubstratesalsoseemsinterestingbecause from the data shownitisclearthattheyhave the ability to modulate the entry of saltinto the plant. In particular, the EM microorganismsthat are probablyable to stimulate the plant'shormonalsystem and retainsodium, guaranteethat the plant can overcomethistype of stress.

For bothzeolites and microbial-basedproducts, itisimportant to underlinethat the use of quality and certifiedmaterialsis of fundamentalimportance to obtainhomogeneous and repeatableresults.

Substrates with addedchabazitic-zeolites and effectivemicroorganisms can thereforenotonlyimprove the qualitative characteristics of plants, butalso reduce the effect of certainabioticstresses, in this case that of the presence of NaCl.Thisaspectcould be particularlyinteresting for thoseareaswhereplants are grown with water with a richcontent of salts, aschabazitic-zeolites and Emcould mitigate this stress.

## V. CONCLUSION

These trials showed several benefits that can be obtained through the use of chabazitic-zeolites and Effective microorganisms: improvement of quality in *Euphorbia milii* and *Crassulaovata* plants, in terms of vegetative and radical growth, better use of fertilizers and water, mitigation of the effects caused by the presence of NaCl.

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**Table**

**Table 1** - Effect of the addition of NaCl on the growth of *Euphorbiamilii*plants

| Treatment | Plantheight (cm) | Leaves number (n°) | Flowersnumber (n°) | Total freshplantweight (g) |
|---|---|---|---|---|
| **CTRL** | 10.22 ± 1.22 c | 14.02 ± 1.66 c | 8.11 ± 0.72 c | 18.60 ± 0.45 c |
| **T1** | 16.13 ± 1.00 b | 18.22 ± 1.04 b | 20.14 ± 0.94 b | 24.33 ± 0.68 b |
| **T2** | 23.50 ± 0.96 a | 24.06 ± 0.56 a | 31.08 ± 0.48 a | 32.55 ± 0.67 a |

Eachvaluereported in the graphis the mean of threereplicates ± standard deviation. Statistical analysisperformedthroughone-way ANOVA. Differentletters for the sameparameter indicate significantdifferencesaccording to LSD test (P = 0.05).

**Table 2** - Effect of the addition of NaCl on the growth of *Crassula ovata*plants

| Treatment | Plantheight (cm) | Leaves number (n°) | Total freshplantweight (g) |
|---|---|---|---|
| **CTRL** | 8.13 ± 0.46 c | 16.13 ± 1.11 c | 29.44 ± 0.63 c |
| **T1** | 13.21 ± 0.56 b | 22.33 ± 1.42 b | 35.31 ± 0.76 b |
| **T2** | 19.53 ± 0.32 a | 28.05 ± 0.98 a | 41.50 ± 0.54 a |

Eachvaluereported in the graphis the mean of threereplicates ± standard deviation. Statistical analysisperformedthroughone-way ANOVA. Differentletters for the sameparameter indicate significantdifferencesaccording to LSD test (P = 0.05).

**Figures**

**Fig**.1 –Differences in the growth of the rootsystem of Euphorbia(a) and Crassula (b)depending on the differentsubstrate and the addition of NaCl

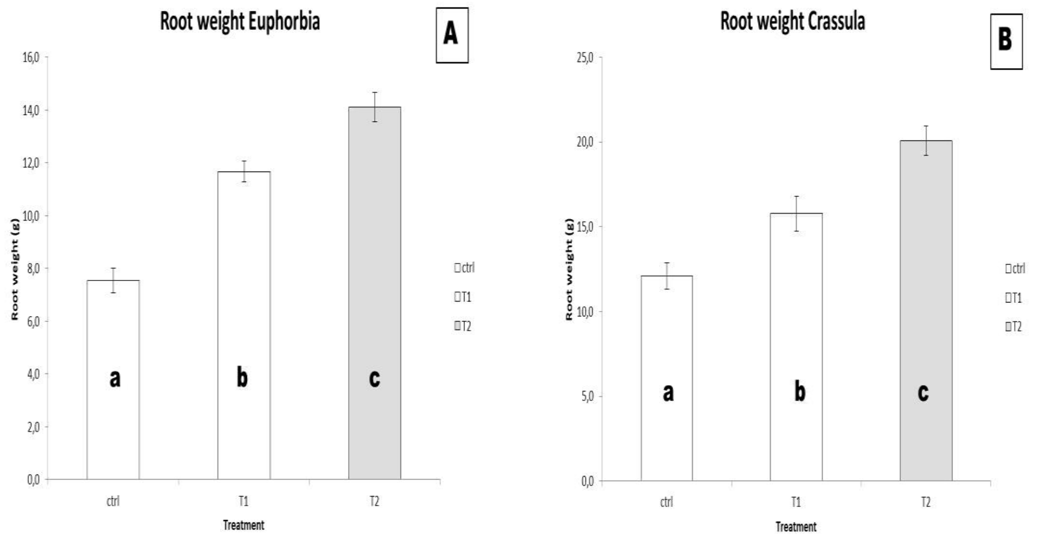
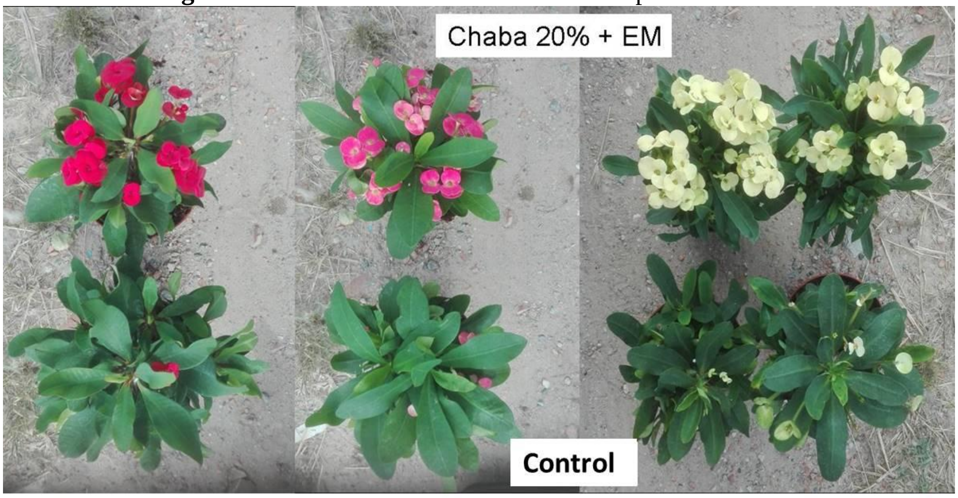



**Fig.2** – Effect of innovative substrates on Euphorbiaflowers

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