



(RESEARCH ARTICLE)



Effective microorganisms for germination and root growth in *Kalanchoe daigremontiana*

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Abstract

The aim of the experiment was to evaluate the use of effective micro-organisms (EM) as promoters of root development and germination of *K. daigremontiana* plants. The 3 experimental groups in cultivation were: 1) group without micro-organisms (CTRL), irrigated with water and previously fertilised substrate; 2) group with sugar cane molasses (ML) and fertilised substrate; 3) group with effective micro-organisms (EM) and fertilised substrate. The test showed a significant increase in the agronomic parameters analysed in plants treated with effective micro-organisms. In fact, all plants treated with (EM) showed a significant increase in plant height, number of leaves, vegetative and radical weight, number and weight of new shoots, leaf area. Also regarding the physiological analysis of plants there is a significant increase in the parameters analyzed in the thesis with effective micro-organisms. In particular, there was a significant increase in the chlorophyll content, in leaf net photosynthesis rate (Pn) and water use efficiency (WUE) and an equally significant reduction in transpiration (TR) and stomatological conductance (Gs). In the thesis treated with EM, the experiment also showed an increase in the percentage of seed germination and a significant reduction in the average germination time. Effective micro-organisms can be a valuable tool for grafting before planting new plants, for spraying and irrigation of crops, for reducing or eliminating completely the use of plant protection products against pathogens and diseases, during the storage of plant raw materials and to increase the biological activity of the soil.

Keywords: Succulent; Plant growth; Rhizobacteria; Rhizosphere; Ornamental plants

1. Introduction

Kalanchoe daigremontiana is a plant of the Crassulaceae family, native to south-east Madagascar where it grows on the hillside of Mount Androhibolava. It is a very robust plant, with an erect posture, simple stem or little branched, up to one meter high. The leaves, up to 20 cm long, slightly toothed at the margins, have a shiny green or bronzed upper page, while the lower one is greyish green, irregularly spotted with dark green, brown. On the edges of the leaves, between one tooth and the other, come out adventitious propagules which, falling on the ground, originate new small plants which root immediately. The flowers, grey-purple, tubular and up to 2 cm long, bloom by the end of winter in the adult plants. This plant reproduces forming many small helicoidal-shaped seedlings, on the edge of each of the fleshy and triangular leaves; when these seedlings fall, they effectively root even on small portions of soil. This *Kalanchoe* is very easy to grow, possibly in full sunlight on a fertile, but well drained substratum. It must be watered abundantly in the growing season, ie. spring-summer, while during the autumn and winter the plant is to be left almost dry. The too old plants tend to get rid of the leaves at the base, and it is good that they are periodically renewed by uprooting them. Due to its climatic collocation, the *Kalanchoe daigremontiana* is not particularly resistant to the cold, and it is therefore advisable to keep it at a temperature not lower than 10 degrees. However, it has been verified that the plant can easily bear even lower temperatures, around the 5 °C, if not irrigated and kept in conditions of not excessive atmospheric humidity [1]. Being a short day plant, like many tropical species, cultivated in apartment, it blooms only if placed in an

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environment where it is not affected by the artificial lighting. *K. daigremontiana* could be a plant toxic and harmful to the native flora and fauna [2,3,4,5] and there is also evidence that it can modify some soil properties [6], but its effects on the cycle of elements such as C, N and P are still unknown.

Effective micro-organisms are a commercial microbial selection containing a mixture of coexisting beneficial micro-organisms collected from the natural environment.

This selection was developed at the University of Ryukyus, Japan, in early 1980 by Prof. Dr. Terou Higa. About 80 different microorganisms are able to positively influence the decomposing organic substance in such a way as to transform it into a process of "promoting life".

The main species involved in EM include [7,8]:

- Lactic acid bacteria – *Lactobacillus plantarum*, *L. casei*, *Streptoccus lactis*;
- Photosynthetic bacteria – *Rhodopseudomonas palustris*, *Rhodobacter sphaeroides* [9];
- Yeast – *Saccharomyces cerevisiae*, *Candida utilis*;
- Actinomycetes – *Streptomyces albus*, *S. griseus*;
- Fermenting fungi – *Aspergillus oryzae*, *Mucor hiemalis*.

EM is a fermented mixed culture of naturally occurring species of micro-organisms coexisting in an acid environment (pH less than 3,5). Micro-organisms in EM improve crop health and yield by increasing photosynthesis, producing bioactive substances such as hormones and enzymes, accelerating the decomposition of organic materials and controlling soil diseases. Effective micro-organisms can be used as herbal insecticides to control insects and pathogenic microorganisms and can also be used as plant growth inducers. Soil micro-organisms have an important influence on soil fertility and plant health. EMs interact with the soil-plant ecosystem by controlling plant pathogens and disease agents, solubilising minerals, increasing plant energy availability, stimulating the photosynthetic system, maintaining the microbiological balance of the soil, fixing biological nitrogen [10]. A characteristic of this mixture is the coexistence of aerobic and anaerobic micro-organisms. After Higa's research in Japan [7], the characteristics of EM have been studied in many countries. Studies have shown positive effects of the application of EM on soils and plants on soil quality and nutrient supply [11], plant growth [12,13,14], crop yield [15,11] and crop quality [16,17,18]. However, in some studies no positive effects were found [10,19,20].

The beneficial effects of micro-organisms introduced with the application of composts, animal manure and compost tea are often short lived leaving crop plant exposed to soil associated conditions. On application, EM mixtures are also subjected to the same conditions in the soil environment. However, the main advantage the effective microorganisms have over natural organisms in organic amendments is that in EM, beneficial micro-organisms are in much greater numbers, optimally-balanced populations when introduced. They would therefore persist in the soil environment for a much longer time enough to bring about the beneficial effects. Studies have shown that, not only does the use of effective micro-organisms in agricultural soil suppress soil-borne pathogens, but also increases the decomposition of organic materials and consequently the availability of mineral nutrients and important organic compounds to plants [21]. In addition, EM enhances the activities of beneficial indigenous micro-organisms, for example mycorrhizae which fix atmospheric nitrogen thereby supplementing the use of chemical fertiliser and pesticides. Improvement in soil fertility has significant positive effect on plant growth, flowering, fruit development and ripening in crops [22].

In this study, the possibility of using effective micro-organisms (EM) as root growth and development improvers in *K. daigremontiana* plants was evaluated.

2. Material and methods

2.1. Greenhouse experiment and growing conditions

The experiments, which began in early April 2019, were conducted in the CREA-OF greenhouses in Pescia (Pt), Tuscany, Italy (43°54'N 10°41'E) on plants of *Kalanchoe daigremontiana* (Figure 1). The plants were placed in pots ø 12 cm; 60 tense plants, divided into 3 replicas of 20 plants each. All plants were fertilized with a controlled release fertilizer (3 kg m⁻³ Osmocote Pro® 6 months with 190 g/kg N, 39 g/kg P, 83 g/kg K) mixed with the growing medium before transplanting. The 3 experimental groups in cultivation were:

- group without micro-organisms (CTRL), irrigated with water and previously fertilised substrate;
- group with sugar cane molasses (ML) and fertilised substrate;
- group with effective micro-organisms (EM) and fertilised substrate, dilution 1:100 (1L of EM inoculum dilution 1:100 was used for each 10L of peat, same proportion for the control thesis where only water was used).

The same proportion was also applied to the treatment with sugar cane molasses alone. During the experiment, 4 treatments with EM and molasses were carried out. The plants were watered 3 times a week and cultivated for 6 months. Plants were irrigated by drip irrigation. Irrigation was triggered by a timer whose schedule was adjusted weekly according to climate condition and leaching fraction. On September 20, 2019, the leaves number, the vegetative and radical weight, the number and weight of new shoots, the leaf area, seed germination and average germination time were recorded. Ten days before the destructive analysis, the chlorophyll content (FieldScout CM 1000 Chlorophyll Meter; SPAD index was measured on two leaves of each plant (360 measurements per treatment), net photosynthesis (Pn), transpiration (Tn) and water use efficiency (WUE) (LI-6400XT Portable Photosynthesis System) were evaluated.

2.2. Statistics

The experiment was carried out in a randomized complete block design. Collected data were analysed by one-way ANOVA, using GLM univariate procedure, to assess significant ($P \leq 0.05, 0.01$ and 0.001) differences among treatments. Mean values were then separated by LSD multiple-range test ($P = 0.05$). Statistics and graphics were supported by the programs Costat (version 6.451) and Excel (Office 2010).

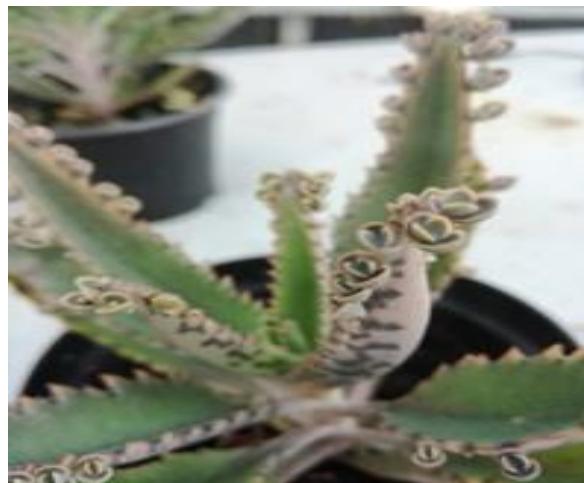


Figure 1 Details of the *Kalanchoe daigremontiana* plant

3. Results

3.1. Plant growth

The test showed a significant increase in the agronomic parameters analysed in plants treated with effective micro-organisms. In fact, all plants treated with (EM) (Table 1) showed a significant increase in plant height (Figure 2), number of leaves, vegetative and radical weight (Figure 3B), number and weight of new shoots (Figure 3A), leaf area. In *Kalanchoe daigremontiana*, the height of the plants was 17.62 cm (EM), compared to 15.98 cm (ML) and 15.80 cm (CTRL). For the number of leaves, 17.42 (EM), 13.42 (ML) and 11.52 (CTRL) were found. There was a significant increase in vegetative biomass in (EM) 96.34 and 76.50 g (ML), compared with 61.48 g in the untreated control. The same trend applies to the root weight 54.74 g in (EM), 41.24 g in (ML) and 38.47 g (CTRL). The thesis with effective micro-organisms then showed a significant increase in the number of new shoots, 110.58 (EM) versus 87.42 (ML) and 70.08 in the untreated control. Also for the weight of the new shoots there is a significant increase in the treatment with (EM) 1.17 g, against 0.76 g of (ML) and 0.57 g of the control. There was also a significant increase in leaf area in the thesis with EM, 250, 33 $\text{cm}^2 \text{pt}^{-1}$, compared to 197.11 $\text{cm}^2 \text{pt}^{-1}$ (ML) and 194.56 $\text{cm}^2 \text{pt}^{-1}$ (CTRL).

Table 1 Evaluation of the effect of effective micro-organisms (EM) on growth of *Kalanchoe daigremontiana*

Groups	Height plant (cm)	Leaves number (n°)	Veg. weight (g)	Root weight (g)	Number new shoots (n°)	Weight new shoots (g)	Leaf area (cm ² pt ⁻¹)
CTRL	15,80 ^b	11,58 ^c	61,48 ^c	38,47 ^b	70,08 ^c	0,57 ^c	194,56 ^b
ML	15,98 ^b	13,42 ^b	76,50 ^b	41,24 ^b	87,42 ^b	0,76 ^b	197,11 ^b
EM	17,62 ^a	17,42 ^a	96,34 ^a	54,78 ^a	110,58 ^a	1,17 ^a	250,33 ^a
ANOVA	***	***	***	***	***	***	***

One-way ANOVA; n.s. – non significant; *, **, *** – significant at $P \leq 0.05$, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test ($P = 0.05$)

Also regarding the physiological analysis (**Table 2**) of plants there is a significant increase in the parameters analyzed in the thesis with effective microorganisms. In particular, there was a significant increase in the chlorophyll content, 12.55 (EM) compared to 11.11 (ML) and 10.12 of the untreated control. In the thesis treated with effective micro-organisms, there is also a significant increase in Leaf net photosynthesis rate (Pn) and Water use efficiency (WUE) and an equally significant reduction in transpiration (Tr) and stomatological conductance (Gs). In the thesis treated with Effective micro-organisms, the experiment also showed an increase in the percentage of seed germination and a significant reduction in the average germination time (**Table 3**).

Table 2 Evaluation of the effect of effective micro-organisms on the physiological characteristics of *Kalanchoe daigremontiana*

Groups	Chlorophyll content (spad index)	Pn (μmol m ⁻² s ⁻¹)	Tr (mmol m ⁻² s ⁻¹)	Gs (mol m ⁻² s ⁻¹)	WUE (μmol CO ₂ /mmol H ₂ O)
CTRL	10,12 ^c	9,72 ^c	2,96 ^b	3,03 ^a	3,30 ^b
ML	11,11 ^b	10,63 ^b	3,23 ^a	2,47 ^b	3,33 ^b
EM	12,55 ^a	12,10 ^a	2,00 ^c	1,16 ^c	6,13 ^a
ANOVA	***	***	***	***	***

One-way ANOVA; n.s. – non significant; *, **, *** – significant at $P \leq 0.05$, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test ($P = 0.05$). Legend: Leaf net photosynthesis rate (Pn), chlorophyll (SPAD index) content, stomatal conductance (Gs), transpiration (Tr), water use efficiency (WUE).

Table 3 Evaluation of the effect of effective micro-organisms in the germination of *Kalanchoe daigremontiana*

Groups	Seed germination (%)	Average germination time (days)
CTRL	87,33	16,00 ^b
ML	88,10	15,00 ^b
EM	94,60	10,00 ^a
ANOVA	-	***

One-way ANOVA; n.s. – non significant; *, **, *** – significant at $P \leq 0.05$, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test ($P = 0.05$).

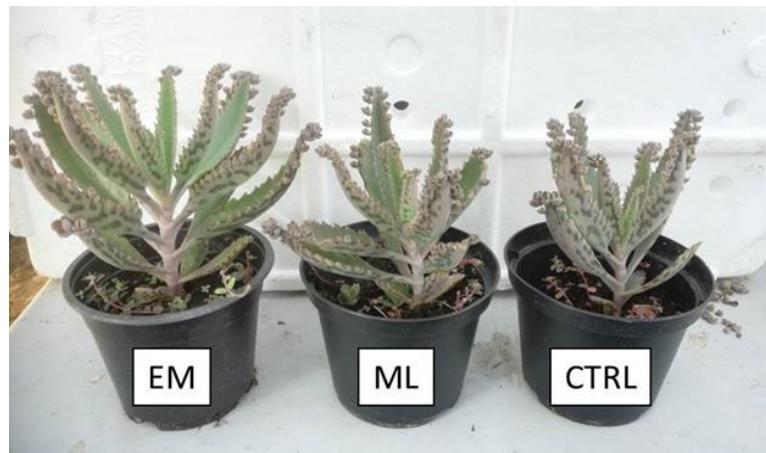


Figure 2 Effect of effective micro-organisms on growth of *Kalanchoe daigremontiana*.
Legend: (EM) effective micro-organisms; (ML) molasses; (CTRL) control.

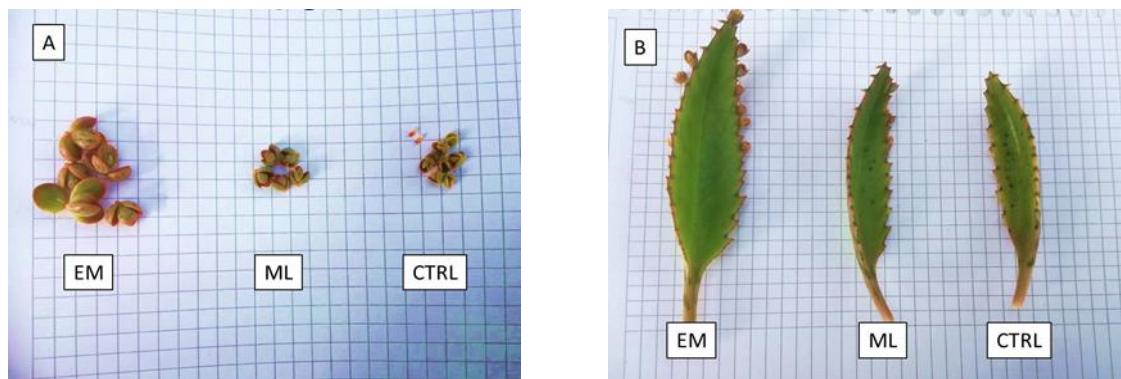


Figure 3 Effect of effective micro-organisms and molasses on growth of new shoots (A) and leaves (B).
Legend: (EM) Effective micro-organisms; (ML) molasses; (CTRL) control.

4. Discussion

The literature does not reveal studies on the effects of effective micro-organisms on the quality of *Kalanchoe daigremontiana* plants, although several works show the effects of this microbial selection on horticultural, ornamental and fruit crops [23,24,25].

In this test, plants treated with (EM) showed a significant increase in plant height, number of leaves, vegetative and radical weight, number and weight of new shoots, leaf area.

The EM micro-organisms determine an increase of the radical development that influences the absorption of water and nutrients. This is reflected in the vegetative development of the plants, which grow faster and produce more new shoots. Increased root development also results in increased resistance to water and transplant stress [26].

The results show a faster growth rate of EM-treated *Kalanchoe* seedlings and a reduction in the possible development of fungal diseases, particularly in nurseries in the early stages of growth.

Scientists have shown that effective micro-organisms can increase fruit weight, yield and photosynthesis as also found in this experiment. In particular, EM applied with compost and manure significantly increased tomato yields, which in the third year of cultivation were comparable to those obtained with chemical fertilizers [10,13,14].

The use of EM has reduced in some cases the colonization of roses from different varieties of aphids: *Macrosiphum rosae* L., *Macrosiphum euphorbiae* (Thom.), *Chaetosiphon tetrarhodium* (Walk.), *Metopolophium dirhodum* (Walk.), *Maculolachnus submacula* (Walk.), *Myzaphis rosarum* (Kalt.), *Aphis fabae* Scop.[27,28].

Effective micro-organisms can be a valuable tool for grafting before planting new plants, for spraying and irrigation of crops, for reducing or eliminating completely the use of plant protection products against pathogens and diseases, during the storage of plant raw materials and to increase the biological activity of the soil [13,16,17].

5. Conclusion

The test has shown how the use of effective micro-organisms can improve the quality and development of *Kalanchoe daigremontiana*, in particular by significantly increasing plant height, number of leaves, vegetative and radical weight, number and weight of new shoots, leaf area. There was also a marked improvement in leaf net photosynthesis rate (Pn) and water use efficiency (WUE) and an equally significant reduction in transpiration (TR) and stomatological conductance (Gs). In the thesis treated with Effective micro-organisms, the experiment also showed an increase in the percentage of seed germination and a significant reduction in the average germination time.

The application of EM in agricultural processing guarantees higher standards, with a significant reduction in production costs. The products obtained from the cultivation with Em have a high antioxidant potential, very important especially in this historical period where food also acts as a medicine.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares no conflict of interest.

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