

‘Effective Micro-organisms’ (EM): An Effective Plant Strengthening Agent for Tomatoes in Protected Cultivation

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ABSTRACT

The effect of treating organically grown tomato plants with Effective Micro-organisms (EM) combined with a stone dust-suspension (EM treatment) was tested in a pot experiment in a foliar tunnel at the University of Natural Resources and Life Sciences Vienna. In the EM treatment, the irrigation water was amended with EMa[®] and plants were treated with EM-stone dust-suspension. In the control treatment, tap water was used instead. In the EM treatment, bokashi, wheat bran fermented with EMa[®], was additionally added to the planting substrate in both years. Only in 2007, the equivalent amount of wheat bran, composted without EMa[®] addition, was added to the substrate in the control as well. Inorganic N contents of the substrate were lower in the control in 2006, but increased when wheat bran compost was added in 2007. N mineralization at later stages of the experiment was higher in the EM treatment in 2007. Microbial biomass in the substrate was enhanced in both years. Total yield was higher and the number of fruits damaged by blossom-end rot was reduced in the EM-treated plants in 2007. The percentage of fruits in the best quality class was significantly higher in the EM treatment in both years. N, P and K contents in tomato leaves of the EM treatment were reduced, whereas the Fe content was higher. A more even N supply to the plants in the EM treatment, combined with the effect of a direct stone dust-application onto the plants, clearly increased plant yield and fostered plant health.

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INTRODUCTION

The sustainability of plant production is related to soil fertility. Improving soil fertility therefore contributes to a sustainable production of healthy plants. The preparation, Effective Micro-organisms (EM), originates from Japan and is increasingly applied in organic and sustainable farming. This product was developed by T. Higa, Ryukyus University, Okinawa, in the early 1980s. The main micro-organisms in EM are lactic acid bacteria (*Lactobacillus plantarum*, *L. casei*, *Streptococcus lactis*), yeasts (*Saccharomyces cerevisiae*, *Candida utilis*), photosynthetic bacteria (*Rhodospseudomonas palustris*, *Rhodobacter spaeroides*), actinomycetes (*Streptomyces albus*, *S. griseus*) and fungi (*Aspergillus oryzae*, *Mucor hiemalis*) (Daly & Steward 1999; Higa, 2002). A characteristic of this mixture is the coexistence of aerobic and anaerobic micro-organisms. The preparation is sold as a stimulator of substrate mineralization and plant growth (Higa & Parr, 1994) and is also applied as "bokashi". Bokashi is the result of a fermentation of organic substance, e.g. wheat bran, with addition of EM. Applying EM is not a substitute for other agricultural strategies but rather an additional dimension to optimize the practices and productivity in organic farming (Higa, 2004).

Initiated by the research of Higa in Japan (Higa, 2004), the potential of EM has been studied in many countries. Some studies showed or indicated positive effects of EM application onto soils and plants on the quality and nutrient delivery of the soils (Aylesworth, 1979; Xu, 2001), on plant growth (Aylesworth, 1979), on crop yield (Xu, 2001), and on crop quality (Higa, 2004). In some studies, however, no positive effects were found (Formowitz *et al.*, 2007; Mayer *et al.*, 2008; Schenck zu Schweinsberg-Mickan and Müller, 2009).

The present study tested the effects of a combined EM-stone dust-application as a means of plant strengthening in sheltered organic tomato production. The nutrient contents and micro-organisms in the substrate, the nutrient contents in tomato leaves, and plant development, plant yield and quality were examined.

MATERIALS AND METHODS

This experiment was conducted according to organic farming regulations in a foliar tunnel (30 × 9 m) with automatic ventilation in the experimental garden of the Institute of Horticulture and Viticulture in Vienna, Austria. Tomato cultivars were Cassiopeia in 2006 and Mercedes in 2007. The plants were pre-cultivated and planted into pots with a volume of 12 l at 45 and 50 days after sowing in 2006 and 2007, respectively. The pots were arranged in ten randomized blocks with eight pots each in the two treatments.

Plants were grown in organic potting soil (Edaphos® e-Mix = Edaphos 2 + Mest-Best, an organic fertiliser from rhizinus grist, clay minerals, seaweed and biodynamic preparations) of the company Josef Gerner, Austria. In the combined EM-stone dust-treatment (short: EM treatment), bokashi, i.e. wheat bran fermented with EMa®, was added to the potting soil at a ratio of 13 g kg⁻¹. In the control treatment, pure potting soil without addition of bokashi/composted wheat bran was used as substrate in 2006. In 2007, an equivalent amount of wheat bran compost without addition of EMa® was added to the potting soil in the control treatment. In that year, therefore, the only difference in the substrate between the two treatments was the addition of EMa®.

Plants in the EM treatment were continuously irrigated with irrigation water amended with 0.33% EMa® in 1.5 l per pot by a hand sprayer each day during the first 31 days after sowing. The untreated control plants were irrigated with EMa®-free tap water. A mixture of EMa®, EM5, EM-FPE, and stone dust (Biolit) was sprayed onto the plants at a concentration of 15.0, 0.3, 0.3 and 1.2 g l⁻¹ water, respectively, every 3 weeks, in total seven times during the growing period. Control plants were watered and sprayed with tap water only.

Nutrient contents of the substrates at the beginning of the experiment after bokashi addition were compared (Table 1). Inorganic N in the substrate, C and N mineralization and microbial biomass were assessed three times after planting (Table 2).

Analysis of total N (N_t) followed ÖNORM L 1095 (2009). Inorganic N in a 0.01 M CaCl₂ extract was assessed according to ÖNORM L 1091 (2009). Nitrate was measured in a UV-VIS photometer at 210 nm (Navone, 1964) regarding the background of organic substance as a blank, and ammonium based on a colour reaction at 660 nm (Krom, 1980).

Plant available P and K contents in calcium acetate lactate extracts (P_{CAL} and K_{CAL}) were assessed only in 2007 (ÖNORM L 1087, 2009). The analytical

TABLE 1

N, P and K contents of the substrates at tomato planting, 45 (2006) and 50 (2007) days after sowing

Nutrients	2006		2007	
	EM	CO	EM	CO
N _t (%)	1.08	1.04	0.99	0.95
P _{CAL} (µg g ⁻¹)	834	850	871	795
K _{CAL} (µg g ⁻¹)	3693	3892	3883	3901

EM: EM-stone dust-treatment; CO: control; N_t: total N; P_{CAL}: plant available phosphorus in calcium-acetate-lactate (CAL) extracts. K_{CAL}: plant available potassium in CAL extracts.

procedure was the same as for nutrient contents in plant leaves given below.

C and N mineralization of the substrates were assessed at the first sampling date, 51 days after sowing, in 2006, and at the third date, 146 day after sowing, in 2007. N mineralization was estimated in vitro according to a standardized procedure (Kandeler, 1993). Soil samples (1–3 g) were covered with water and incubated anaerobically for 7 days at 40°C. Ammonium was measured as described above. The difference in ammonium contents between incubated samples and non-incubated control samples is a measure for N mineralization. C mineralization was measured according to Jäggi (1976). The substrate samples were incubated in a sealed vessel for seven days. The CO₂ evolved was trapped in NaOH and quantified by titration.

Microbial biomass carbon and nitrogen in the substrate were assessed by the chloroform fumigation extraction method (Brookes *et al.*, 1985; Vance *et al.*, 1987) at the first sampling date in 2006 and at the first and third sampling dates in 2007. C and N extracted with 0.5 M K₂SO₄ solution from fumigated and non-fumigated samples were analysed by a Dimatoc 100 TOC-TNb analyzer.

Nutrients in plant leaves were analysed at two dates in 2007: 1. in fully developed leaves at the beginning of flowering, 96 days after sowing, 2. at full insemination, 128 days after sowing. N content in leaves was measured by Kjeldahl digestion. The other nutrients (P, K, Mg, Ca, Fe, Mn, Cu und Zn) were extracted with a mixed acid (10 parts HNO₃, 1 part H₂SO₄, 0.5 parts HClO₄) from the dried leaves. P was measured on a photometer at 436 nm (Gericke and Kurmies, 1952). The other elements were measured on an atom absorption spectrometer.

To determine the germination rate, the appearance of seed leaves was counted on 125 seeds per treatment each day during the first 21 days after sowing. When the first fruits started ripening, all marketable and mature fruits were harvested two times a week, counted and weighed in each repetition plot (balance Präzisa, Zurich, Switzerland). Tomato quality was classified according to various characteristics. Tomatoes were divided into two commercial categories: class extra (= tomato of the highest quality, with typical varietal form and appearance, free from greenbacks with very slight superficial skin defects) and class 1 (= tomato of medium quality, with healed cracks and visible green collar and patches or unusual form). Unmarketable fruits were also counted and classified in different categories of damage. The average fruit weight was calculated by dividing the weight of all harvested marketable fruits by the number of fruits.

Results of the two years were separately analysed statistically in an ANOVA. The two factors treatment and block were tested. The homogeneity of variance of the residuals was tested and fulfilled.

RESULTS

Nitrogen, phosphorus and potassium in the substrate before sowing

The contents of N_p , P_{CAL} and K_{CAL} in the substrate did not differ clearly between treatments in either year (Table 1). No statistical test was applied here because substrates were not replicated. Only P_{CAL} contents in the EM treatment were slightly higher than those of the control treatment in 2007. Wheat bran had been added to both treatments in that year and cannot account for any differences in nutrient contents.

Inorganic nitrogen in the substrate

Inorganic N in the substrate was measured at three dates (Table 2). In both years, a significant treatment effect was found only at the first date. The ammonium content was significantly higher in the EM treatment in 2006 due to the EM bokashi addition to the potting soil (Table 2). In 2007, when wheat bran compost was added to the control treatment, the inorganic N content, mainly nitrate content, was significantly lower in the EM treatment (Table 2).

Carbon and nitrogen mineralization in the substrates

C mineralization was significantly higher in the EM treatment than in the control in both years ($p < 0.05$) (Table 2). N mineralization rates on average did not differ significantly between the EM treatment and the control in 2006, significant differences being difficult to demonstrate owing to high and varying ammonium contents (Table 2). In 2007, N mineralization was enhanced at 146 days after sowing in the EM treatment. This result and lower inorganic N contents in the EM treatment at the beginning of the experiment (Table 2) indicate a retarded N release upon EM bokashi addition to the substrate compared to the control where wheat bran compost was added.

Microbial carbon and nitrogen

In 2006, microbial C contents were significantly higher in the EM treatment, whereas microbial N contents did not differ significantly. In 2007, both microbial C and N were significantly increased in the EM treatment at the first date, whereas only microbial N was increased at the third date (Table 2).

Plant available phosphorus and potassium

Contents of plant available P_{CAL} and K_{CAL} in the substrate were assessed at the three main dates in 2007. P_{CAL} was lower at the first, K_{CAL} at the first and second date in the EM treatment (Table 2).

Germination

In both years, plants germinated earlier and seeds germinated at a higher rate in the EM treatment (Figure 1).

Mineral element contents in tomato leaves

N, P and K contents in tomato leaves were lower and Fe contents were higher in the EM treatment at the first assessment date in 2007. At the second date, N, P and K contents no longer differed, whereas Fe and Mn contents were higher in the EM treatment (Table 3).

Fruit yield and quality

The fruit yield was higher in the EM treatment ($P < 0.05$) in 2007 (Table 4). In 2006, yield did not differ significantly. In both years the percentage of fruits in the best quality class extra was significantly increased in the EM treatment, whereas the average fruit weight of all harvested fruits did not differ between the treatments. In 2007, the percentage of damaged fruits affected by blossom-

TABLE 3

Mineral nutrient contents in tomato leaves in 2007.

	96 days after sowing		128 days after sowing	
	EM	CO	EM	CO
N (%)	21.2 ± 1.8 a	27.1 ± 0.7 b	12.4 ± 0.6 a	13.4 ± 1.3 a
P (%)	0.07 ± 0.01 a	0.08 ± 0.01 b	0.00 ± 0.00 a	0.00 ± 0.00 a
K (%)	3.6 ± 0.3 a	3.93 ± 0.19 b	1.59 ± 0.11 a	1.80 ± 0.26 a
Mg (%)	0.1 ± 0.02 a	0.14 ± 0.02 a	0.06 ± 0.01 a	0.06 ± 0.00 a
Ca (%)	0.42 ± 0.03 a	0.42 ± 0.01 a	0.40 ± 0.01 a	0.41 ± 0.04 a
Fe ($\mu\text{g g}^{-1}$)	840 ± 93 a	183 ± 21 b	977 ± 126 a	318 ± 163 b
Mn ($\mu\text{g g}^{-1}$)	56.6 ± 8.1 a	47.4 ± 8.2 a	58.3 ± 4.6 a	42.0 ± 1.0 b
Cu ($\mu\text{g g}^{-1}$)	30.4 ± 7.3 a	32.5 ± 8.9 a	23.8 ± 0.6 a	23.7 ± 0.7 a
Zn ($\mu\text{g g}^{-1}$)	67.5 ± 5.2 a	63.2 ± 3.2 a	22.4 ± 2.1 a	27.9 ± 1.6 a

EM: EM-stone dust-treatment; CO: control; – not determined. Mean value ± standard deviation; mean values at one date with the same letter do not significantly differ (ANOVA, $p < 0.05$).

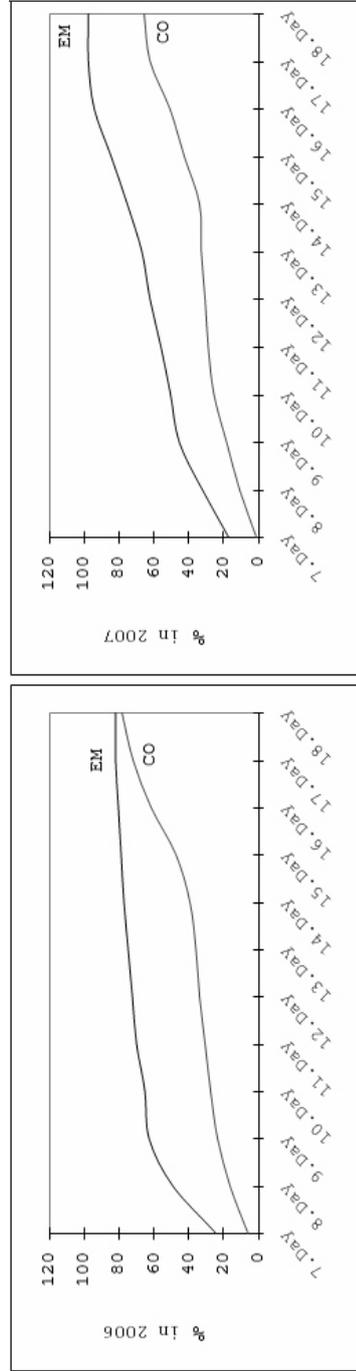


FIGURE 1. Rate and period of germination of 125 tomato seeds per treatment in 2006 (left) and 2007 (right). For legend see Table 2.

TABLE 4

Yield and quality classes of tomatoes at harvest.

	2006		2007	
	EM	CO	EM	CO
Total weight of healthy fruits (g per plant)	6630 ± 479 a	5889 ± 1009 b	3107 ± 281 a	2243 ± 190 b
Quality class extra (%)	75.0 ± 18.3 a	44.8 ± 21.7 b	65.6 ± 20.2 a	54.2 ± 28.2 b
Quality class 1 (%)	25.1 ± 19.1 b	55.2 ± 88.1 a	34.4 ± 22.3 b	45.8 ± 25.2 a
Number of healthy fruits per plant	59.4 ± 4.3 a	56.8 ± 5.7 b	30.2 ± 2.5 a	23.5 ± 1.4 b
Average fruit weight of all harvested fruits (g per fruit)	141 ± 12.4 a	135 ± 32.0 a	103 ± 15.7 a	95.6 ± 21.4 a

EM: EM-stone dust-treatment; CO: control; – not determined. Mean value ± standard deviation; mean values at one date with the same letter do not significantly differ (ANOVA, $p < 0,05$).

end rot, a physiological disorder due to calcium deficiency, was significantly higher on the untreated plants (Figure 2).

DISCUSSION AND CONCLUSIONS

The effect of a combined EM-stone dust-application on yield, nutrient content and quality of tomato plants in sheltered cultivation was tested. The EM treatment can, on the one hand, alter the nutrient availability in the substrate by the addition of EM bokashi. On the other hand, watering with EM solution and spraying with EM-stone dust-suspension can supply the plants with metabolic substances from EM and nutrients from the stone dust. Both possible effects were evaluated.

EM effect on nutrient availability

The amount of EM bokashi or wheat bran compost added to the potting soil (13 g kg^{-1}) was moderate. Adding EM bokashi did not increase the contents of N_t , P_{CAL} or K_{CAL} in the substrate at the beginning of the experiment in 2006 (Table 1). Only inorganic N contents were increased (Table 2). Adding wheat bran compost in the control treatment also did not increase N_t , P_{CAL} or K_{CAL} contents in the substrate at the beginning of the experiment in 2007 compared with the control without compost addition in 2006. Inorganic N contents at the first date were likewise increased. Accordingly, both EM bokashi and wheat bran compost functioned as fertilizers with a high N, and a low P and K availability.

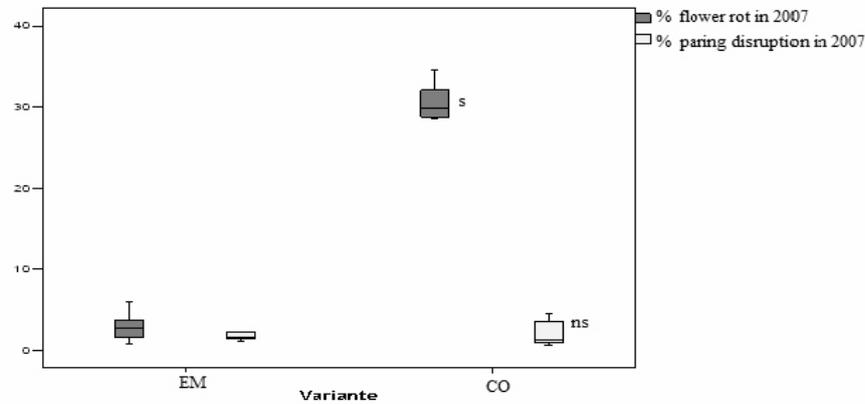


FIGURE 2. Fruits affected by blossom-end rot and paring disruption in % of all fruits in 2007. For legend see Table 2. s: treatment effect significant ($p < 0.05$); ns: treatment effect not significant. Boxes represent 50 % percentiles, bars represent 25% and 75% percentiles.

As a result of EM bokashi addition, ammonium contents, C mineralization rate and microbial biomass were enhanced compared with the control without compost addition at the first date in 2006 (Table 2). This reflects a high substrate turnover rate by a larger microbial biomass in the EM treatment at this date. In 2007, inorganic N and P_{CAL} contents decreased and microbial C and N in the EM treatment increased compared with the control (wheat bran compost added) at the first date, and C and N mineralization rates increased at the third date (Table 2). This indicates a larger and more active microbial population in the EM treatment, clearly temporarily immobilizing N and P. The microbial inoculum in the compost therefore affected the nutrient turnover and availability in the substrate. Yamada & Xu (2000) also reported effects of an EM inoculation of organic fertilizers on the quality of fermented products. A significant positive effect of EM on plant production has been found only or mainly when EM was applied in combination with organic substances like crop residues or farmyard manure (Cho *et al.*, 1999, Hussain *et al.*, 1999, Khaliq *et al.*, 2006). EM treatment increased the efficiency of nutrient supply by organic and mineral fertilizers (Khaliq *et al.*, 2006). Nishio (1995) concluded that vegetables fertilized with bokashi needed the same amount of nitrogen as when they were given chemical fertilizers. This implies an availability of nitrogen in bokashi equivalent to that in mineral fertilizer. Schenck zu Schweinsberg-Mickan & Müller (2009), on the contrary, found no effect of EM application on nitrogen mineralization rates and a suppressive effect on soil microbial biomass in a pot experiment.

The above-mentioned enhanced activity of an increased microbial biomass after EM bokashi was added to the substrate in 2007 reduced the plant availability of main nutrients temporarily. This is reflected by reduced contents

of N, P, and K in tomato leaves at 96 days after sowing (Table 3). This effect did not last until the next assessment at 128 days after sowing (Table 3). The temporarily reduced nutrient (mainly N) availability and uptake by the plants in this study, however, was not disadvantageous. An abundant N supply can increase the susceptibility to bacterial and fungal diseases (Mengel, 1991). The enhanced N mineralization rate at a later stage (in 2007: 146 days after planting, Table 2) signifies a more evenly distributed N delivery in the EM treatment.

Other studies reported increased nutrient contents upon EM treatment in bananas (Formowitz *et al.* 2007), rice and wheat (Tahir *et al.*, 1999) and sugar maize (Xu, 2001), but not a temporally reduced nutrient availability. Positive EM effects on nutrient uptake were related to an enhanced root growth of EM-treated plants (Xu, 2001). An enhanced colonization by mycorrhiza in EM-treated plants (Rukhsana *et al.*, 1999) can help improve nutrient uptake. Root growth and mycorrhizal colonization were not assessed in this study.

Micro-nutrient contents of the leaves were assessed in 2007. The Fe and Mn contents were higher in the EM treatment (Table 3). Although an increased uptake from the EM bokashi treated substrate cannot be excluded, presumably the EM-stone dust-suspension sprayed onto the leaves was the main cause. Stone dust contains micro-nutrients like Fe and Mn. The application together with EM, which, among other substances, produces organic acids like lactic acid, can promote the release and uptake of these nutrients by leaves.

EM effect on plant yield and quality

Plant yield on average was increased. In 2006, when no wheat bran was added to the control, the resulting difference in the amount of supplied nutrients is the main cause of any effects on yield and nutrient availability. In 2007, however, nutrient in the substrates did not differ. Still, differences in nutrient release and availability occurred that were clearly favourable in the EM treatment. In line with this result, Jenkins and Daly (2005) found a furthered plant development only with EM bokashi treatment, not for bokashi without EM. Other studies also showed positive yield effects of EM treatment in maize (Marambe *et al.*, 1999: EM sprayed; Daly & Steward 1999: EM plus molasses; Xu, 2001: EM plus bokashi), beans (Sangakkara *et al.*, 2008: EM plus cattle manure), onions (Chamberlain *et al.*, 1999; Daly & Steward, 1999: EM plus molasses) and tomato (Xu *et al.*, 2000a: EM plus bokashi). Mayer *et al.* (2008) and Schenck zu Schweinsberg-Mickan & Müller (2009), however, found no effect on plant growth in a field experiment with potato and in a pot experiment with *Lolium perenne*, respectively.

Plant quality, i.e. the percentage of fruits in quality class extra, was improved in the EM treatment in both years (Table 4). Xu *et al.* (2000a), for example,

found higher vitamin C contents in EM-treated tomatoes. In total, plant health was better in the EM treatment, as related to the number of healthy fruits and the percentage of fruits with blossom-end rot (Figure 2). The latter can be related to a less abundant N supply along with increased Fe and Mn contents of plant leaves during plant youth compared to the control, because micro-nutrient deficiency favours blossom-end rot (Oehmichen, 1983; Vogel, 1996; Bedlan, 1999).

Beyond the already mentioned positive effects involving a more evenly distributed N and P supply, an improved supply with Fe and Mn, and a possibly enhanced root growth, the observed positive effect of the EM treatment on yield, plant quality and health can have additional causes. EM solutions contain bioactive substances. Borgen (1998), for example, found an equally reducing effect of living and autoclaved EM solutions against common bunt (*Tilletia tritici*) and interpreted this as an effect of metabolic substances in the EM solution, e.g. lactic acid. Xu *et al.* (2000b) found an EM effect on stomatal response in maize and interpreted this as an effect of bioactive substances produced by EM. Therefore, a missing effect of a treatment with living EM versus a sterilized, autoclaved control cannot be interpreted as proof of an ineffectiveness of EM treatment.

EM treatment can also affect the physico-chemical traits of the soil or substrate. It can reduce crusting and improve aggregation and drainage, changing overall the biological equilibrium and the living conditions of pathogens (Tokeshi *et al.*, 1997). To what extent this effect was significant in our study cannot be deduced from our results. In studies with successful results after EM application, the importance of a systemic EM application is emphasized, i.e. application via compost/bokashi, via the soil, and via a direct treatment of the plant at the same time (Cho *et al.* 1998), over a long period of time and a sufficiently large area (Marambe *et al.*, 1998; Sangakkara *et al.*, 2000). This leads to the assumption that EM have to establish in the soil plant system and then unfold their effects through several mutually supporting pathways. This may also explain why EM effects are more frequently found under applied, field-level conditions than in exact scientific trials that usually test only single factors in smaller-scale experiments. A colonization of the rhizosphere and the plants with EM as endophytes seems likely but has not yet been tested.

Adding EM bokashi to the potting soil stimulated the microbial population and activity in the substrate compared with a control without bokashi addition or a control with an addition of wheat bran compost without EM. This enhanced microbial activity temporarily reduced plant availability of the main nutrients N, P and K. The combined EM application by addition of EM to the substrate as bokashi, by watering the plants and by spraying them with EM-stone dust-suspension increased the yield and improved the health of tomato plants. More research is required to determine the mechanisms by which EM

influence plant growth and health, including the mineralization of nutrients from the stone dust that was applied together with EM.

ACKNOWLEDGEMENT

We thank Andreas Lössl, Department of Applied Plant Sciences and Plant Biotechnology, for analysis of nutrient contents in tomato leaves. This study was supported by Multikraft®, Austria.

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