MICRONUTRIENTS BIOFORTIFICATION IN SPINACH (*SPINACIA OLERACEA*) USING URBAN WASTE COMPOST INOCULATED WITH DIFFERENT *TRICHODERMA* SPECIES

BIOFORTIFIKACIJA ŠPINATA MIKROHRANIVIMA KORISTEĆI KOMPOST URBANOG OTPADA INOKULIRAN RAZLIČITIM TRICHODERMA VRSTAMA

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***Abstract**: The purpose of this research was to investigate using of different amount of municipal waste compost (MWC) enriched with 50% chemical fertilizer and inoculated with three Trichoderma species on nutrition uptake of spinach (biofortification) under greenhouse conditions. The experiment was carried out as factorial arrangement in a randomized complete block design with three replications. Results indicated that aerial Zn and Cu contents were highest in the 45 and 30 Mg ha^{-1} MWS compost. Also, the 45 Mg ha^{-1} compost caused highest Mn accumulation. The soil inoculation with Trichoderma hamatum increased significantly aerial Mn content compared to T. harzianum and T. viridi (12.84% and 12.48%, respectively).*

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***Key words**: spinach, biofortification, Trichoderma sp, nutrition, urban*

***Sažetak:**Svrha ovog istraživanja bila je ispitati kako se mijenjaju hranjive karakteristike špinata uzgajanog u stakleniku, koristeći različite količine komunalnog otpada obogaćenog s 50% kemijskog gnojiva, cijepljenog s tri Trichoderma vrste.*

***Ključne riječi:** špinat, biofortifikacija, Trichoderma vrsta,hranjiv*

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1. Introduction

Compost is commonly defined as the aerobically stabilized or matured organic matter, although anaerobic processes can also lead to the production of a stabilized (or matured) organic material. Municipal waste compost and other biosolids are a valuable source of some macro and micro nutrients and organic matter. Incorporating biosolids improves the physical properties, biological activity, and fertility of soils [4][5]. Most of the nutrients in this organic amendment are found in forms available to plants and are released into the soil in a slow manner [9].

Clearly, previous experimental researches aiming to specifically addressing interactions between organic amendments applications, micro organisms and their consequences for crop plants seems necessary [1][3].

Trichoderma species can improve plant growth and development. Growth stimulation is evidenced by increases in biomass, productivity, stress resistance and increased nutrient absorption [2]. In the present work, the objective was to determine the efficacy of biosolids and beneficial fungi of the rhizosphere in some micronutrients accumulation of the spinach (*Spinacia oleracea*).

2. Material and Methods

¶2.1 Strains

Three Trichoderma species used for the investigation were *T. harzianum* Rifai, *T. viridi* and *T. hamatum* Bon. Cultures were maintained at 24°C on Potato Dextrose Agar (PDA, Difco) for four days, and liquid Potato Dextrose broth (PDB) for 72 h at a speed of 120 rpm for sporulation. Spores were collected on filter paper in a Buchner funnel, washed with distilled water and adjusted with sterilized distilled water in 108/mL colony forming units (cfu). Trchoderma spores were adjusted with sterilized distilled water in 108 mL colony forming units (cfu) for 1 Kg/dry weight of media pot.

2.2 General Description

Soil and municipal waste compost characteristics at the beginning of the experiment are shown in Table 1. These soils originated from silty and clay mineral parent material (Typic haploxerept: clay, 44%, silt, 46% and sand, 10%).

2.3 Study design and plant material

The experiment was carried out as factorial (2^2) arrangement in a randomized complete block design with three replication in plastic pots (20 cm diameter). The treatments consisted of 15, 30 and 45 t. ha^{-1} of municipal solid waste compost (MSWC) separated and enriched with 1/2 chemical fertilizer, alone chemical fertilizer (100 Kg ha^{-1} of urea, potassium sulfate and triple super phosphate) and untreated soil (compost or chemical fertilizer) inoculated with three Trichoderma species (T. *harzianum, T. hamatum and T. viridi*).

2.4 Plant Measurements and Laboratory Analysis

The metal contents (Fe, Zn, Cu, Mn) of spinach leaf were determined by Atomic Absorbtion Spectrometery (Spectra aa –Australia) after calcinations of the sample (0.5 g) at 550 ºC and solution of the ashes in concentrated HCL (38%) and further dilution to 50 cm3.

2.5 Statistical Analysis

All the data were analyzed statistically with the computer software SPSS and subjected to ANOVA. Comparison among treatment means was done by the Duncan’s Multiple Range Test (DMRT) multiple-comparison test.

3. Tables

Characteristic[a]	N (%)	P	K	Mn	Cu	Zn	Fe	C:N ratio	pH
		mg/kg							
Soil	0.23	14.56	278.05	13.96	5.57	1.02	58.47	10.95	7.52
MSW	0.53	45	8485.76	251.96	362.18	766.39	7154.81	11.14	7.41

Table 1. Chemical properties of the soil and municipal solid waste (MSW) compost used in the study

[a]Data refer to dry matter (105 ºC) of surface soil (0–30 cm) sieved at < 2 mm.

4. Results

Micronutrient concentrations in the aerial part of spinach were significantly altered by fertilizer treatments and different between Trichoderma species (Table 2). In most cases, the micronutrient concentrations of plant aerial part were significantly enhanced by increasing of MWS compost in the substrate.

All of organic amendments were caused significantly increases in Zn, Cu and Fe concentration compared to CF and control treatments. Aerial Zn and Cu contents were highest in the 45 and 30 Mg ha-1 MWS compost, respectively.

Plant iron content was maximum in the 30 Mg ha-1 compost and 45 Mg ha-1 compost (enriched and non-enriched) also, the 45 Mg ha-1 compost (enriched and non-enriched) caused highest Mn accumulation (Table 2). In the previous study shown that sequential applications of compost with and without fertilizer increased the nutrient uptake of parsley[7].

Concentrations of Zn, Cu, Mn and Mo were determined to be the highest in plant tissue from the compost treatment reflecting their high concentrations in the compost material. Enhancing soybean growth and increasing plant nutrition uptake (Mn, Cu, Fe and Zn) reported by using of organic fertilizers (sewage sludge, municipal waste compost and vermicompost) which enriched with chemical fertilizer [6].

Treatments	Zn (mg Kg^{-1})	Cu (mg Kg^{-1})	Fe (mg Kg^{-1})	Mn (mg Kg^{-1})
Fertilizer (T)				
MSW45+1/2 CF	121.22b	28.84b	101.32a	99.41a
MSW45	130.40a	27.55bc	107.43a	95.18ab
MSW30+1/2 CF	108.75c	28.55b	91.08b	89.52bc
MSW30	112.26b	32.74a	99.38a	85.64cd
MSW15+1/2 CF	96.19d	25.26cd	77.30c	77.38de
MSW 15	106.84c	26.66bc	75.14cd	75.00e
CF	85.60e	23.36de	67.52ef	65.77ef
C	81.33e	22.07e	62.01e	59.66f
Fungi (F)				
T. harzianum	104.53ab	26.71ab	79.86b	77.05b
T. viridi	102.73b	27.83a	82.48b	77.37b
T. hamatum	108.70a	26.09b	93.11a	88.41a
Significance				
T	**	**	**	**
F	NS	NS	**	**
T*F	NS	NS	NS	NS

Table 2. Mean comparison of some studied traits in different fertilizer amounts and fungus
* Means with in the same column followed by the same letters were not significantly to DMRT (P<0.05).

5. Conclusion

In this current experiment, the soil inoculation with Trichoderma hamatum increased significantly aerial Mn content compared to *T. harzianum* and *T. viridi* (12.84% and 12.48%, respectively). Also, results showed that 11.41% and 14.23% increases of Fe aerial concentration in *T. hamatum* soil inoculated versus *T. harzianum* and *T. viridi*, respectively (Table 2).

Öğüt and Er (2006) showed that T. harzianum generally decreased Fe, Mn, Zn, and Cu in bean foliar biomass but significantly increased Cu in the seeds. In these same trials, *T. harzianum* also increased Mn accumulation in the seeds of wheat. In cucumber plants, inoculation with *T. harzianum* caused a significant increase in P, Cu, Fe, Zn, Mn and Na concentration in the roots, which was accompanied by a 25%, 30% and 70% increase in Zn, P, and Mn, respectively, in the shoots [8].

6. References

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Photo 144. Cottage on Papuk / Koliba na Papuku