

Evaluation of Phytotoxicity of Organic Acids by Germination Test

Ocjena fitotoksičnosti organskih kiselina testom klijavosti

Lončarić, Z., Galić, L., Nikolin, I., Nemet, F., Perić, K.

Poljoprivreda / Agriculture

ISSN: 1848-8080 (Online)

ISSN: 1330-7142 (Print)

<https://doi.org/10.18047/poljo.32.1.5>



Fakultet agrobiotehničkih znanosti Osijek, Poljoprivredni institut Osijek

Faculty of Agrobiotechnical Sciences Osijek, Agricultural Institute Osijek

EVALUATION OF PHYTOTOXICITY OF ORGANIC ACIDS BY GERMINATION TEST

Lončarić, Z.,⁽¹⁾ Galić, L.,⁽¹⁾ Nikolin, I.,⁽¹⁾ Nemet, F.,⁽¹⁾ Perić, K.⁽²⁾

Original scientific paper

Izvorni znanstveni članak

SUMMARY

Phytotoxicity, regardless of the cause, can significantly reduce plant growth and development from seed germination onwards. Therefore, the germination test is the most widely used laboratory method for determining phytotoxicity, and garden cress and cucumber are most commonly used as test plants. The aim of this study was to determine the phytotoxic and/or phytostimulatory effect of different concentrations of acetic (AA), lactic (LA), butyric (BA) and citric (CA) acids. In the germination test, garden cress and cucumber seeds were exposed to different concentrations (0, 0.1, 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 mM) of the above organic acids in order to determine and compare the concentrations with phytotoxic effect. The results show that garden cress is more suitable for determining the effect on germination and high phytotoxicity, and cucumber for determining moderate phytotoxicity. All tested acids had a phytotoxic effect at certain concentrations. The least toxic was LA, slightly more toxic were CA and AA, and BA was the most toxic, especially for garden cress. The most stimulating effects on garden cress were at lower or medium concentrations, but the stimulating effect on cucumber was almost completely absent. Simultaneous testing by germination test with garden cress and cucumber is a justified combination due to their different reactions to organic acids, and such a germination test is recommended for testing the phytotoxicity of all composts and substrates, especially immature ones.

Keywords: germination test, phytotoxicity, germination index, acetic acid, butyric acid, citric acid, lactic acid

INTRODUCTION

Unregulated waste management systems lead to the accumulation of various compounds that can be phytotoxic, in addition to contaminating soil and water (Chandra et al., 2005). Phytotoxicity is a relatively common occurrence in agricultural practices, both due to pesticide residues and due to increased salinity or phytotoxicity of organic soil additives, like immature compost. Immature compost may contain various phytotoxic substances like heavy metals (Tam and Tiquia, 1994), phenols (Wong, 1985), ethylene, ammonia (Tam and Tiquia, 1994, Lončarić et al., 2024a), salts (Tiquia and Tam, 1998), and organic acids (Manios et al., 1989) that can inhibit seed germination and plant growth (Lončarić et al., 2024a). Organic acids are naturally occurring compounds with important roles in plant metabolism,

soil chemistry, microbial activity, and soil biochemical processes, but under certain conditions, plant roots can secrete various organic acids into the rhizosphere that inhibit plant growth (Rice, 1984).

Chandrasekaran and Yoshida (1973) reported that the decomposition of fresh organic matter residues can produce acetic, propionic, butyric, and isobutyric organic acids, which can have phytotoxic effects. In poorly drained soils with anaerobic conditions, the decomposition of fresh organic matter produces phytotoxic substances (Camargo et al., 2001, Barral and Paradelo,

(1) Prof. Dr. Zdenko Lončarić (zdenko.loncagic@fazos.hr), Lucija Galić, PhD, Iva Nikolin, M. Eng. Agr., Franjo Nemet, M. Eng. Agr. – Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia, (2) Katarina Perić, M. Eng. Agr., Agricultural Institute Osijek, Južno predgrađe 17, 31000 Osijek, Croatia

2011), among which organic acids such as butyric, acetic and propanoic acids stand out, and the toxic effect of these acids on the plant depends on the type and concentration of the organic acid (Himanen et al., 2012, Rao and Mikkelsen, 1977). In general, concentrations of organic acids can vary from tens to hundreds of mg/kg of soil and are about 5 - 10 times higher in plant residues (Goh et al., 1986). Ulbright et al. (1982) state that carboxylic acids of lower molecular weights are known in agriculture as crop growth inhibitors. Baumgarten and Spiengel (2004) state that phytotoxicity causes reduced germination, inhibits plant growth and development, and has other adverse effects caused by certain phytotoxins or inadequate and inappropriate growing conditions.

Phytotoxicity is best assessed by germination or growth testing (Gariglio et al., 2002., Brewer and Sullivan, 2003). Germination tests are widely applied in agricultural research (Zucconi et al., 1985), and among test species used in germination assays, garden cress (*Lepidium sativum* L.) and cucumber (*Cucumis sativus* L.) are particularly suitable because of their rapid germination and high, but different, sensitivity to phytotoxic or phytostimulating compounds (Lončarić et al., 2024b). Among the organic acids most frequently investigated in germination studies are acetic, citric, lactic, and butyric acids because of their occurrence in decomposing organic matter and agricultural substrates.

Acetic acid is a short-chain organic acid whose phytotoxic effects depend on concentration (Camargo et al., 1993). At low concentrations, it may have no negative effect, while higher concentrations strongly inhibit germination and root elongation. Acetic acid is often produced during anaerobic decomposition of organic matter and is important in assessing soil phytotoxicity and compost maturity.

Citric acid and lactic acid are considered less phytotoxic than acetic or butyric acid. Several studies have shown that moderate concentrations of citric or lactic acid have minimal negative effects on germination or can even improve seed germination and seedling growth, but excessive concentrations can reduce germination, inhibit radicle emergence, and affect seedling growth.

One of the most phytotoxic organic acids for seed germination is butyric acid because of strong inhibition of germination, root elongation, and seedling development even at relatively low concentrations. Butyric acid is formed during anaerobic decomposition of organic matter and is often an indicator of compost immaturity. Particularly sensitive to butyric acid is garden cress, which is used as a standard test species in assessing the phytotoxicity of compost, but cucumber is also significantly inhibited by butyric acid. Due to its strong inhibitory effects, butyric acid is often considered a reliable indicator of anaerobic toxicity in organic substrates.

The aim of this research was to determine the phytotoxicity of organic acids and to determine the concentration at which seed germination and root growth inhibition occur. The aim was also to determine whether there are certain concentrations of these acids that have

a phytostimulating effect and whether there is a difference between the reactions of garden cress and cucumber to different concentrations of the acetic, lactic, citric, and butyric acids.

MATERIAL AND METHODS

Germination Test

The evaluation of the phytotoxic or phytostimulative effect of solutions of 4 different organic acids was carried out using a modified germination test method (Zucconi et al., 1981a), which is commonly used for compost testing, and the authors used the method to evaluate the phytotoxicity of immature compost. The modified method combines germination and root growth, and shoot emergence and length of test plants (Zucconi et al., 1981b). The germination test was conducted using two test plants, garden cress (*Lepidium sativum* L.) and cucumber (*Cucumis sativus* L.).

The germination test examined the effect of 4 organic acids on garden cress and cucumber: (1) acetic acid ($C_2H_4O_2$), (2) lactic acid ($C_3H_6O_3$), (3) butyric acid ($C_4H_8O_2$), and (4) citric acid ($C_6H_8O_7$), using 8 different concentrations of the mentioned organic acids (including deionized water as a control treatment, that is, 0.0 mM concentration treatment).

Solutions of organic acids were prepared by weighing a certain mass of the acid and dissolving it in deionized water to a volume of 1,000 ml as a stock solution. By pipetting a certain volume of the stock solution of each organic acid, solutions of 0.00 (deionized water as control), 0.10, 0.25, 0.50, 1.00, 2.00, 4.00, and 8.00 mM acetic, lactic, butyric, or citric acid were prepared. Garden cress and cucumber seeds were prepared for germination testing by washing 2,500 seeds of each test species according to the prescribed procedure. Petri dishes were also prepared with filter paper placed at the bottom. In each Petri dish, 5 mL of the appropriate solution (deionized water or a solution of a certain concentration of a particular organic acid) was placed on filter paper, and 20 garden cress or cucumber seeds were carefully placed on the filter paper. Each treatment was set up in 4 replicates, meaning that 116 Petri dishes were set up for each species (112 for 7 concentrations of each organic acid + 4 for the control treatment with deionized water), or a total of 232 in the entire experiment. The Petri dishes were placed at 25°C under controlled conditions, and after 72 hours (3 days), the planned measurements were taken: the number of germinated seeds and the length of the roots and shoots of each individual plant. germinated rate (GR) as a percentage of germinated seeds, root length per plant (RLP), root length index (RI), germination index (GI), shoot rate (SR) as a percentage of visible shoots, shoot length per plant (SLP), shoot length index (SI), and a vitality shoot index (MLSV).

GR represents the percentage of germinated seeds out of a total of 20, and is based on the number of seeds recorded with visible beginnings of radicle development.

RLP, as the average length in cm of all radicles of germinated seeds, was calculated by adding up the radicle lengths of each individual plant in one Petri dish and dividing by the number of germinated seeds:

RLP [Root Length per Plant] = $\sum RL_{1-NGS} / NGS$ (NGS = number of germinated seeds) [cm].

RI represents a comparison of the average root length of each individual repetition of a particular treatment with the average root length of the control treatment (deionized water) and is expressed in % relative to the control treatment:

RI [Root Index] = $[(RL_{s1}/RL_c + RL_{s2}/RL_c + RL_{s3}/RL_c + RL_{s4}/RL_c)/4] \times 100$ [%].

GI was calculated as the product of two ratios, the ratio of the number of germinated seeds of the treatment to the control, and the ratio of the average root length of the treatment to the control, and is expressed as an index according to EN 16086-2 (EN, 2012):

GI [Germination Index] = (Germinated seeds / Germinated seeds in control) \times (RLP_s/RLP_c).

SR represents the percentage of seeds out of a total of 20, and is based on the number of seeds recorded with visible beginnings of coleoptile (shoots) development.

SLP as the average length in cm of all shoots, was calculated by adding up the shoot lengths of each individual plant with a visible shoot in one Petri dish and dividing by the number of seeds with a visible shoot:

SLP [Shoot Length per Plant] = $\sum SL_{1-NSS} / NSS$ (NSS = number of seeds with visible shoot) [cm].

SI represents a comparison of the average shoot length of each individual repetition of a particular treatment with the average shoot length of the control treatment (deionized water) and is expressed in % relative to the control treatment:

SI [Shoot Index] = $[(SL_{s1}/SL_c + SL_{s2}/SL_c + SL_{s3}/SL_c + SL_{s4}/SL_c)/4] \times 100$ [%].

Munoo–Liisa vitality index [MLSV] or the vitality shoot index, is an index calculated using SR and SL and a modified formula according to EN 16086-2 (EN, 2012):

MLSV = $[(SR_{s1} \times SL_{s1}) + (SR_{s2} \times SL_{s2}) + (SR_{s3} \times SL_{s3}) + (SR_{s4} \times SL_{s4})] / [4 \times (SR_c \times SL_c)] \times 100$ [%].

Statistical Data Processing

Statistical analyses of data were performed with MS Excel software package and SAS for Windows 9.1.3. (SAS Institute Inc., Cary, NC, USA). Levene's test for homogeneity of variance was first step in statistical data

analysis, and afterwards, analysis of variance (ANOVA) with Fisher's least significant difference (LSD) test was performed.

RESULTS AND DISCUSSION

Germination rate (GR) in the control treatment (deionized water) was 95% for garden cress and cucumber (Tables 1-8).

Lower concentrations of organic acids, from 0.1 to 1.0 mM, did not significantly reduce the germination of garden cress (Table 1, 3, 5 and 7), with the exceptions of 0.1 mM CA and 0.25 mM LA (but still with relatively high GR (81,25 and 77.5%). Two organic acids with the mildest effect on garden cress germination were LA (GR = 85-87.5% at 2.0- 8.0 mM) and CA (GR = 83.75% at 4.0 mM), with a more intensive reduction of garden cress germination only at 8.0 mM CA (GR = 56.25%).

The most intensive reduction in garden cress GR was caused by BA, with a decrease to 58.75% already at 2.0 mM, and without any germinated seeds (GR = 0) at the highest concentration (8.0 mM). AA had a similar effect, since at 8.0 mM no seeds germinated (GR = 0), but GR was statistically significantly reduced only at a concentration of 4.0 mM AA.

None of the tested organic acids at the highest concentration (8.0 mM) significantly reduced cucumber germination (GR = 85-90%, Table 2, 4, 6, and 8). Also, LA did not have a statistically significant effect on cucumber germination at all (Table 4). CA and BA had a practically similar modest effect, although GR was still statistically significantly lower at 0.1 mM and 1.0 mM CA (81.25%, Table 6) and 0.25 mM BA (78.75%, Table 8). Finally, AA had the most intense effect on cucumber germination (Table 2) because all concentrations reduced GR (although at 1.0 and 8.0 mM without statistical significance), with the lowest GR (67.5%) being at 4.0 mM AA.

The effect of AA on garden cress and cucumbers was different, with more intense and regular patterns of effect on garden cress than on cucumbers. Germination of garden cress was very significantly reduced at an AA concentration of 4.0 mM, and seeds did not germinate at all at 8.0 mM (Table 1). On the other hand, cucumber germination was lowest at an AA concentration of 4.0 mM, statistically significantly lower than in the control sample, also at concentrations of 0.10, 0.50, and 2.0 mM (Table 2), so we cannot confirm the regularity of the effect of AA concentration on cucumber germination.

Table 1. Indicators of AA Effect on Garden Cress

Tablica 1. Indikatori utjecaja octene kiseline na kres salatu

Acid / Kiselina mM	GR (%)	RLP (cm)	RI (%)	GI	SR (%)	SLP (cm)	SI (%)	MLSV (%)
control	95.0 ^A	13.45 ^C	100.00 ^C	1.00 ^C	95.00 ^A	5.21 ^C	100.00 ^C	100.00 ^C
AA 0.10	87.5 ^{AB}	19.51 ^{AB}	144.94 ^B	1.33 ^B	85.00 ^A	6.81 ^{AB}	130.28 ^{AB}	117.38 ^{ABC}
AA 0.25	90.0 ^{AB}	20.99 ^{AB}	155.93 ^{AB}	1.47 ^{AB}	85.00 ^A	4.86 ^{CD}	93.03 ^{CD}	82.12 ^D
AA 0.50	92.5 ^A	24.27 ^A	180.29 ^A	1.75 ^A	92.50 ^A	7.38 ^A	141.20 ^A	137.28 ^{AB}
AA 1.00	92.5 ^A	22.45 ^{AB}	166.80 ^{AB}	1.63 ^{AB}	92.50 ^A	7.75 ^A	148.43 ^A	145.84 ^A
AA 2.00	92.5 ^A	13.59 ^C	100.97 ^C	0.98 ^C	92.50 ^A	5.63 ^{BC}	107.70 ^{BC}	104.53 ^{BCD}
AA 4.00	81.2 ^B	2.30 ^D	17.08 ^D	0.15 ^D	63.75 ^B	3.58 ^D	68.45 ^D	45.59 ^E
AA 8.00	0.0 ^C	0.00 ^D	0.00 ^E	0.00 ^D	0.00 ^C	0.00 ^E	0.00 ^E	0.00 ^F
LSD _{0.05}	9.508	4.057	30.144	0.316	12.791	1.540	29.474	32.942

Letters in superscript (^{A, B}) in table columns indicate significant difference at level $p < 0.05$ /Slova u superskriptu (^{A, B}) u stupcima tablice označavaju značajnu razliku na razini $p < 0,05$

The influence of increasing concentrations of AA on GI of garden cress (Table 1) as the most important property in the phytotoxicity test, and on RLP and RI is very regular and almost identical. A statistically significant increase in RLP, RI and GI were determined at AA concentrations of 0.1 to 1.0 mM (highest values at 0.50 mM), afterwards at a concentration of 2.0 mM RLP, RI and GI were again in the range of control values, and with concentrations of 4.0 and 8.0 they were statistically significantly the least (at 8.0 mM garden cress seeds did not germinate at all). By interpreting GI values, we can conclude that all concentrations of AA from 0.1 to 1.0 had a phytostimulating effect ($GI = 1.33-1.75$), and concentrations with high phytotoxicity were 4.0 mM ($GI = 0.15$) and 8.0 mM ($GI = 0.0$). An increase in phytotoxicity with an increase in the concentration of organic acids was also determined in the research of Camargo et al. (1993) and Himanen et al. (2012).

At the same time, only the concentration of 8.0 mM AA had a significant effect on cucumber, both on GI (0.19, high phytotoxicity), and on RLP and RI (Table 2). The concentration of 1.0 mM ($GI = 1.29$) had a phytostimulatory effect on cucumber, and the difference was significant compared to all other concentrations of AA, but not compared to the control.

Using a regression model with GI values ($GI_{AA-GC} = 1,0964 + 1,7591 \times mM - 1,3267 \times mM^2 + 0,2074 \times mM^3$; $r^2 = 0,9818$; $mM \in [0, 4]$), it was determined that for moderate phytotoxicity to garden cress, a concentration of 2.16 mM AA is required, and for high phytotoxicity 2.44 mM AA. For the same effect on cucumber ($GI_{AA-CU} = 1,3983 - 0,2444 \times mM + 0,0119 \times mM^2$; $r^2 = 0,87678$; $mM \in [1, 4]$), concentrations of 2.21 and 3.18 mM AA are required, although even low concentrations of around 0.45 mM AA can have a phytotoxic effect on cucumber. Himanen et al. (2012) found that the effective concentrations of organic acids in the acute seed germination of cress ranged between 1.9 and 4.2 mM and for ryegrass between 1.8 and 6.4 mM.

The effect of AA on garden cress and cucumber was somewhat more similar if we compare the effects on the shoot. In both indicator plants, the highest value of SLP, SI, and MLSV was determined with a concentration of AA of 1.0 mM, and the lowest with a concentration of 8.0 mM. Thus, the phytostimulatory effect of AA 1.0 mM and high phytotoxicity of AA 8.0 mM on the shoots of garden cress and cucumber are clear. Other concentrations of AA had an effect on the cucumber shoot, mostly in the range of the control treatment. In garden cress, a concentration of 4.0 mM was also phytotoxic to the shoot, while concentrations of 0.1 and 0.5 mM were phytostimulant.

Table 2. Indicators of AA Effect on Cucumbers

Tablica 2. Indikatori utjecaja octene kiseline na krastavac

Acid / Kiselina mM	GR (%)	RLP (cm)	RI (%)	GI	SR (%)	SLP (cm)	SI (%)	MLSV (%)
control	95.0 ^A	13.61 ^{AB}	100.00 ^{AB}	1.00 ^{AB}	83.75 ^A	3.00 ^{ABC}	100.00 ^{ABC}	100.00 ^{AB}
AA 0.10	70.0 ^{DE}	14.67 ^{AB}	107.82 ^{AB}	0.78 ^B	53.75 ^C	4.38 ^{AB}	145.31 ^{AB}	92.33 ^{ABC}
AA 0.25	80.0 ^{BCD}	12.76 ^B	93.81 ^B	0.77 ^B	57.50 ^{BC}	3.17 ^{ABC}	105.11 ^{ABC}	64.36 ^{BCD}
AA 0.50	77.5 ^{CDE}	13.98 ^{AB}	102.74 ^{AB}	0.84 ^B	58.75 ^{BC}	4.55 ^A	150.91 ^A	102.48 ^{AB}
AA 1.00	90.0 ^{AB}	18.32 ^A	134.62 ^A	1.29 ^A	71.25 ^{AB}	4.68 ^A	155.28 ^A	131.19 ^A
AA 2.00	77.5 ^{CDE}	12.37 ^B	90.93 ^B	0.74 ^B	60.00 ^{BC}	2.50 ^{BC}	82.84 ^{BC}	54.46 ^{CD}
AA 4.00	67.5 ^E	14.09 ^{AB}	103.57 ^{AB}	0.72 ^B	58.75 ^{BC}	4.15 ^{AB}	137.50 ^{AB}	92.08 ^{BC}
AA 8.00	85.0 ^{ABC}	2.86 ^C	20.99 ^B	0.19 ^C	62.50 ^{BC}	1.33 ^C	44.10 ^C	32.67 ^D
LSD _{0,05}	12.373	5.106	37.531	0.330	17.26	1.933	64.105	39.005

Letters in superscript (^{A, B}) in table columns indicate significant difference at level $p < 0.05$ /Slova u superskriptu (^{A, B}) u stupcima tablice označavaju značajnu razliku na razini $p < 0,05$

The effect of LA was significantly different on garden cress and on cucumber. The effect on garden cress was similar to the effect of AA, with the highest GI, RI, and RLP at a concentration of 1.0 mM LA, but also a phytostimulatory effect on garden cress roots at all LA concentrations, except for the highest concentration of 8.0 mM, with high phytotoxicity (Table 3). The difference in the effect of LA and AA on garden cress is the phytostimulatory effect of a concentration of 4.0 mM LA, but the high phytotoxicity of 4.0 mM AA. Also, significantly lower phytotoxicity of LA than that of the AA was determined at 8.0 mM because at a concentration of 8.0 mM, the LA GR of garden cress was 85.0, and with AA there was no germination of garden cress (GR = 0).

It was determined by a regression model that for moderate phytotoxicity to garden cress ($GI_{LA-GC} = 1,2149 + 0,5503 \times mM - 0,1618 \times mM^2 + 0,0096 \times mM^3$; $r^2 = 0,8402$; $mM \in [0, 8]$), a concentration of 5.9 mM LA is required, and for high phytotoxicity 6.78 mM LA. For the same moderate phytotoxicity effect on cucumber ($GI_{LA-CU} = 0,8665 - 0,3762 \times mM + 0,1136 \times mM^2 + 0,0088 \times mM^3$; $r^2 = 0,6994$; $mM \in [0, 2]$), concentrations of 0,19 mM LA would be required, and for high phytotoxicity concentrations above 8 mM LA.

The phytostimulatory effect of LA on shoots of garden cress was determined at concentrations of 0.5, 1.0, and 4.0 mM, and high phytotoxicity at a concentration of 8.0 mM.

Table 3. Indicators of LA Effect on Garden Cress

Tablica 3. Indikatori utjecaja mliječne kiseline na kres salatu

Acid / Kiselina mM	GR (%)	RLP (cm)	RI (%)	GI	SR (%)	SLP (cm)	SI (%)	MLSV (%)
control	95.0 ^A	13.45 ^D	100.00 ^D	1.00 ^D	95.00 ^A	5.21 ^B	100.00 ^B	100.00 ^B
LA 0.10	92.5 ^{AB}	19.88 ^{BC}	147.72 ^{BC}	1.44 ^{BC}	92.25 ^{AB}	5.60 ^B	107.21 ^B	103.27 ^B
LA 0.25	77.5 ^C	19.37 ^C	143.87 ^C	1.17 ^{CD}	72.50 ^C	6.29 ^B	117.85 ^B	89.42 ^B
LA 0.50	86.2 ^{AB}	24.16 ^{AB}	179.46 ^{AB}	1.62 ^{AB}	83.75 ^B	8.29 ^A	158.72 ^A	138.79 ^A
LA 1.00	92.5 ^{AB}	26.07 ^A	193.64 ^A	1.89 ^A	91.25 ^{AB}	7.87 ^A	150.74 ^A	145.09 ^A
LA 2.00	85.0 ^{BC}	21.79 ^{BCD}	161.90 ^{ABC}	1.44 ^{BC}	85.00 ^B	6.29 ^B	120.42 ^B	107.81 ^B
LA 4.00	87.5 ^{AB}	22.18 ^{BCD}	164.75 ^{ABC}	1.52 ^B	87.50 ^{AB}	7.91 ^A	151.44 ^A	139.29 ^A
LA 8.00	85.0 ^{BC}	2.39 ^E	17.79 ^E	0.16 ^E	75.00 ^C	2.60 ^C	49.76 ^C	36.04 ^C
LSD _{0,05}	9.272	4.476	33.220	0.304	8.717	1.548	29.632	27.839

Letters in superscript (^{A, B}) in table columns indicate significant difference at level $p < 0.05$ /Slova u superskriptu (^{A, B}) u stupcima tablice označavaju značajnu razliku na razini $p < 0,05$

LA had no positive effect on germination, root, and shoot growth of cucumber, since the highest value of all indicators was in the control treatment (Table 4). A significant moderate phytotoxicity on cucumber roots was determined at all LA concentrations starting from 0.50 mM (measured lower values of GI, RLP, and RI). A significant phytoinhibitory effect (phytotoxicity) on cucumber

shoot growth (SLP, SI, and MLSV) was determined at all LA concentrations from 0.10 to 4.00 mM, and it is very interesting that the inhibitory effect on cucumber shoots was absent at the highest LA concentration (8.0 mM), although a moderate phytotoxicity on cucumber roots was simultaneously determined.

Table 4. Indicators of LA Effect on Cucumbers

Tablica 4. Indikatori utjecaja mliječne kiseline na krastavac

Acid / Kiselina mM	GR (%)	RLP (cm)	RI (%)	GI	SR (%)	SLP (cm)	SI (%)	MLSV (%)
control	95.0	13.60 ^A	100.00 ^A	1.00 ^A	83.75 ^A	3.00 ^A	100.00 ^A	100.00 ^A
LA 0.10	86.25	11.67 ^{AB}	85.75 ^{AB}	0.78 ^{AB}	73.75 ^{ABC}	1.94 ^B	64.49 ^B	56.43 ^B
LA 0.25	83.75	11.81 ^{AB}	86.78 ^{AB}	0.78 ^{AB}	63.75 ^C	1.89 ^B	62.59 ^B	48.02 ^B
LA 0.50	82.50	8.76 ^{BC}	64.38 ^{BC}	0.56 ^B	65.00 ^{BC}	1.78 ^B	58.90 ^B	46.04 ^B
LA 1.00	87.50	8.88 ^{BC}	65.24 ^{BC}	0.60 ^B	65.00 ^{BC}	1.81 ^B	60.15 ^B	47.03 ^B
LA 2.00	95.00	7.95 ^C	58.41 ^C	0.58 ^B	77.50 ^{ABC}	1.63 ^B	54.05 ^B	50.00 ^B
LA 4.00	83.75	9.07 ^{BC}	66.69 ^{BC}	0.59 ^B	82.50 ^{AB}	1.26 ^C	41.66 ^C	41.09 ^B
LA 8.00	88.75	9.04 ^{BC}	66.43 ^{BC}	0.63 ^B	72.50 ^{ABC}	2.86 ^A	94.77 ^A	83.17 ^A
LSD _{0.05}	ns	3.185	23.413	0.257	17.577	0.340	11.284	22.038

Letters in superscript (^{A, B}) in table columns indicate significant difference at level $p < 0.05$ /Slova u superskriptu (^{A, B}) u stupcima tablice označavaju značajnu razliku na razini $p < 0,05$

The effect of CA on garden cress was similar to the effect of AA and LA, with slightly more pronounced toxicity, which is already measurable at a concentration of 2.0 mM CA, and high toxicity was determined both at 4 and 8 mM CA (Table 5). The phytostimulative effect on garden cress root was determined at all lower concentrations of CA, from 0.1 to 1.0 mM CA, with the highest effects at 0.5 and 1.0 mM.

Regression modelling determined that a moderate phytotoxic effect of CA on garden cress ($GI_{CA-GC} = 1,3445 - 0,3357 \times \text{mM} + 0,0217 \times \text{mM}^2$; $r^2 = 0,7693$; $\text{mM} \in [0, 8]$) required a concentration of 1.84 mM, and a

high phytotoxic effect of 3.16 mM. A very low concentration of 0.06 mM CA is sufficient for moderate phytotoxicity for cucumber ($GI_{CA-CU} = 0,8902 - 0,4458 \times \text{mM} + 0,0803 \times \text{mM}^2$; $r^2 = 0,8706$; $\text{mM} \in [0, 2]$), and 1.09 mM for high phytotoxicity.

The phytostimulating effect on the shoot of garden cress was determined at concentrations of 0.5 to 4.0 mM CA and slightly less pronounced at 0.1 and 0.25 mM. Strong phytotoxic effect determined at the highest concentration of 8.0 mM.

Table 5. Indicators of CA Effect on Garden Cress

Tablica 5. Indikatori utjecaja limunske kiseline na kres salatu

Acid / Kiselina mM	GR (%)	RLP (cm)	RI (%)	GI	SR (%)	SLP (cm)	SI (%)	MLSV (%)
control	95.00 ^A	13.45 ^{BC}	100.00 ^{BC}	1.00 ^{BC}	95.00 ^A	5.21 ^B	100.00 ^B	100.00 ^B
CA 0.10	81.25 ^B	17.02 ^{AB}	126.46 ^{BC}	1.08 ^{ABC}	92.25 ^{AB}	5.60 ^B	107.21 ^B	103.27 ^B
CA 0.25	90.00 ^{AB}	18.49 ^{AB}	137.38 ^{BC}	1.31 ^{AB}	72.50 ^C	6.29 ^B	117.85 ^B	89.42 ^B
CA 0.50	85.00 ^{AB}	22.75 ^A	129.00 ^A	1.53 ^A	83.75 ^B	8.29 ^A	158.72 ^A	138.79 ^A
CA 1.00	88.75 ^{AB}	20.29 ^A	150.74 ^A	1.41 ^{AB}	91.25 ^{AB}	7.87 ^A	150.74 ^A	145.09 ^A
CA 2.00	90.00 ^{AB}	10.19 ^C	75.71 ^C	0.73 ^C	85.00 ^B	6.29 ^B	120.42 ^B	107.81 ^B
CA 4.00	83.75 ^B	1.92 ^D	14.30 ^D	0.13 ^D	87.50 ^{AB}	7.91 ^A	151.44 ^A	139.29 ^A
CA 8.00	56.25 ^C	2.25 ^D	16.70 ^D	0.10 ^D	75.00 ^C	2.60 ^C	49.76 ^C	36.04 ^C
LSD _{0.05}	11.046	6.332	47.039	0.507	8.717	1.548	29.632	27.839

Letters in superscript (^{A, B}) in table columns indicate significant difference at level $p < 0.05$ /Slova u superskriptu (^{A, B}) u stupcima tablice označavaju značajnu razliku na razini $p < 0,05$

The citric acid, like LA, had no positive effect on cucumber roots; all root growth indicators were lower than control values even at the lowest CA concentrations (Table 6). Root growth indicator values decreased with increasing CA concentration, and phytotoxicity was statistically significant for some indicators starting from 0.50 mM (RLD and RI) or from 1.0 mM (GI) CA concen-

tration. The shoot rate of cucumber was decreased by all concentrations of CA, but SLP, SI, and MLSV were statistically significantly increased starting from 0.5 up to 4.0 mM CA, indicating a phytostimulating effect. In contrast, CA concentrations of 8.0 mM resulted in a distinct phytotoxic effect on the cucumber shoot.

Table 6. Indicators of CA Effect on Cucumbers

Tablica 6. Indikatori utjecaja limunske kiseline na krastavac

Acid / Kiselina mM	GR (%)	RLP (cm)	RI (%)	GI	SR (%)	SLP (cm)	SI (%)	MLSV (%)
control	95.00 ^A	13.60 ^A	100.00 ^A	1.00 ^A	95.00 ^A	5.21 ^B	100.00 ^B	100.00 ^B
CA 0.10	81.25 ^B	11.28 ^{AB}	82.89 ^{AB}	0.73 ^{AB}	92.25 ^{AB}	5.60 ^B	107.21 ^B	103.27 ^B
CA 0.25	91.25 ^{AB}	12.05 ^{AB}	88.55 ^{AB}	0.85 ^{AB}	72.50 ^C	6.29 ^B	117.85 ^B	89.42 ^B
CA 0.50	90.00 ^{AB}	9.52 ^{BC}	69.99 ^{BC}	0.67 ^{ABCD}	83.75 ^B	8.29 ^A	158.72 ^A	138.79 ^A
CA 1.00	81.25 ^B	8.56 ^{BC}	62.91 ^{BC}	0.55 ^{BCD}	91.25 ^{AB}	7.87 ^A	150.74 ^A	145.09 ^A
CA 2.00	93.75 ^{AB}	9.77 ^{ABC}	71.84 ^{ABC}	0.72 ^{ABCD}	85.00 ^B	6.29 ^B	120.42 ^B	107.81 ^B
CA 4.00	83.75 ^{AB}	6.08 ^C	44.68 ^C	0.39 ^D	87.50 ^{AB}	7.91 ^A	151.44 ^A	139.29 ^A
CA 8.00	87.50 ^{AB}	6.76 ^C	49.67 ^C	0.46 ^{CD}	75.00 ^C	2.60 ^C	49.76 ^C	36.04 ^C
LSD _{0.05}	12.704	4.004	29.428	0.337	8.717	1.548	29.632	27.839

Letters in superscript (^{A, B}) in table columns indicate significant difference at level $p < 0.05$ /Slova u superskriptu (^{A, B}) u stupcima tablice označavaju značajnu razliku na razini $p < 0,05$

The effect of BA on garden cress was with even more pronounced toxicity, which is already measurable at a concentration of 1.0 mM BA, and significantly high toxicity was determined at 2.0, 4.0, and 8 mM BA (Table 7). The higher toxicity of butyric acid on garden cress compared to other organic acids was determined in a study by Himanen et al. (2012) and Camargo et al. (1993). The phytostimulative effect on garden cress root was determined at concentrations of 0.1 to 0.5 mM BA, with the highest effects at 0.25 mM (GI = 1,67).

There were no positive effects of BA on SR, and a significant decreasing of SR was determined starting at 2.0 mM BA. However, the phytostimulating effect on the shoot of garden cress was determined at concentrations of 0.1 to 0.5 mM BA, and with no effect at 1.0 mM. A strong phytotoxic effect was determined starting at 2.0 mM and all higher concentrations of BA.

Table 7. Indicators of BA Effect on Garden Cress

Tablica 7. Indikatori utjecaja maslačne kiseline na kres salatu

Acid / Kiselina mM	GR (%)	RLP (cm)	RI (%)	GI	SR (%)	SLP (cm)	SI (%)	MLSV (%)
control	95.00 ^A	13.45 ^{BC}	100.00 ^{BC}	1.00 ^{BC}	95.00 ^A	5.21 ^C	100.00 ^C	100.00 ^A
BA 0.10	91.25 ^A	19.20 ^{AB}	142.64 ^{AB}	1.35 ^{AB}	86.25 ^A	7.62 ^A	145.89 ^A	132.75 ^A
BA 0.25	87.50 ^A	24.32 ^A	180.66 ^A	1.67 ^A	85.00 ^A	6.94 ^{AB}	132.94 ^{AB}	119.40 ^A
BA 0.50	91.25 ^A	14.94 ^{BC}	111.01 ^{BC}	1.07 ^{BC}	87.50 ^A	6.35 ^{ABC}	121.52 ^{ABC}	112.59 ^A
BA 1.00	87.50 ^A	12.10 ^C	89.90 ^C	0.85 ^C	85.00 ^A	5.28 ^{BC}	101.00 ^{BC}	94.71 ^A
BA 2.00	58.75 ^B	2.60 ^D	19.30 ^D	0.12 ^D	18.75 ^B	1.49 ^D	28.56 ^D	5.54 ^B
BA 4.00	36.25 ^C	1.68 ^D	12.46 ^D	0.05 ^D	3.75 ^C	0.50 ^D	9.57 ^D	0.76 ^B
BA 8.00	0.00 ^D	0.00 ^D	0.00 ^D	0.00 ^D	0.00 ^C	0.00 ^D	0.00 ^D	0.00 ^B
LSD _{0.05}	13.893	5,795	43.049	0.421	12.921	1.706	32.661	38.493

Letters in superscript (^{A, B}) in table columns indicate significant difference at level $p < 0.05$ /Slova u superskriptu (^{A, B}) u stupcima tablice označavaju značajnu razliku na razini $p < 0,05$

BA had no significant effect on cucumber RLP or RI, but all the concentrations of BA decreased GI (Table 8), only 0.50 mM without significance, and other effects were at levels of moderate (0,10 to 2.0 mM) or high (4.0 and 8.0 mM) phytotoxicity (Table 8). The SR of cucumber was decreased by all concentrations of BA, and SLP, SA, and MLSV were at the level of control only at a concentration of 0.50 mM BA, while all the other were significantly lower, and lowest at 8.0 mM BA.

Modeling with GI determined that 0.93 mM BA would be sufficient for a moderate phytotoxic effect on garden cress, and 1.57 mM BA for a high phytotoxic effect ($GI_{BA-GC} = 1,4078 - 0,7681 \times mM + 0,1315 \times mM^2 - 0,0072 \times mM^3$; $r^2 = 0,8492$; $mM \in [0, 3]$). The same moderate effects on cucumber would require only 0.9 mM BA, but a high phytotoxic effect 3,07 mM BA ($GI_{BA-CU} = 0,9549 - 0,01834 \times mM + 0,0115 \times mM^2$; $r^2 = 0,9432$; $mM \in [0, 8]$), more than for the same effect on garden cress.

Table 8. Indicators of BA effect on cucumbers

Tablica 8. Indikatori utjecaja maslačne kiseline na krastavac

Acid / Kiselina mM	GR (%)	RLP (cm)	RI (%)	GI	SR (%)	SLP (cm)	SI (%)	MLSV (%)
control	95.00 ^A	13.61 ^A	100.00 ^A	1.00 ^A	83.75 ^A	3.00 ^A	100.00 ^A	100.00 ^A
BA 0.10	87.50 ^{AB}	10.77 ^A	79.13 ^A	0.74 ^B	77.50 ^{AB}	1.36 ^C	44.97 ^C	41.99 ^{CD}
BA 0.25	78.75 ^B	11.32 ^A	83.19 ^A	0.69 ^B	73.75 ^{AB}	1.71 ^C	56.70 ^C	50.00 ^{BCD}
BA 0.50	87.50 ^{AB}	12.75 ^A	93.73 ^A	0.86 ^{AB}	67.50 ^{AB}	3.34 ^A	110.68 ^A	90.10 ^A
BA 1.00	85.00 ^{AB}	11.02 ^A	81.00 ^A	0.73 ^B	72.50 ^{AB}	2.32 ^B	76.74 ^B	66.83 ^B
BA 2.00	91.25 ^A	10.56 ^A	77.63 ^A	0.74 ^B	83.75 ^A	1.70 ^C	56.27 ^C	55.94 ^{BC}
BA 4.00	86.25 ^{AB}	5.30 ^B	38.94 ^B	0.35 ^C	73.75 ^{AB}	1.54 ^C	51.10 ^C	45.05 ^{CD}
BA 8.00	90.00 ^{AB}	3.24 ^B	23.81 ^B	0.23 ^C	61.25 ^B	1.33 ^C	43.99 ^C	34.16 ^D
LSD _{0.05}	12.101	3.242	23.831	0.240	17.92	0.395	13.101	21.371

Letters in superscript (^{A, B}) in table columns indicate significant difference at level $p < 0.05$ /Slova u superskriptu (^{A, B}) u stupcima tablice označavaju značajnu razliku na razini $p < 0,05$

All tested organic acids had, at certain concentrations, a phytostimulating or phytotoxic effect on garden cress, both on the root (according to GI) and on the shoot (according to MLSV). The most stimulating effects of organic acids on garden cress roots were at lower concentrations of 0.25 mM (BA) and 0.5 mM (AA and CA) or medium concentrations of 1.0 mM (LA). Similar, but slightly lower concentrations have the most positive effect on garden cress shoots, because BA has the most stimulating effect at 0.1 mM, and the other acids at 1.0 mM (AA, LA, CA). It is significantly different in cucumber, because the stimulating effect on the root was completely absent (LA, CA, BA), except for AA, which had a stimulating effect only at a concentration of 1.0 mM. Nevertheless, some stimulatory effect on cucumber shoots was determined, which was most positive at a concentration of 1.0 mM (AA and CA), while LA and BA did not have a stimulatory effect.

On the other hand, all acids had a phytotoxic effect at certain concentrations, both on the root and shoot of garden cress and cucumber. GI regression modelling found that lower concentrations were sufficient to produce a moderate toxic effect ($GI < 0.8$) on cucumber roots than on garden cress roots (0.19 vs. 5.90 for LA, 0.06 vs. 1.84 for CA, and 0.90 vs. 0.93 mM for BA), with the exception of AA (2.21 for cucumber vs. 2.16 mM for garden cress), although these two AA concentrations were almost the same. However, the opposite is true with regard to high phytotoxicity ($GI < 0.5$) because in garden cress, 1.57 mM BA, 2.44 mM AA, and 6.78 mM LA are sufficient for high phytotoxicity, while in cucumber, 3.07 mM BA, 3.18 mM AA, and > 8.0 mM LA are required. The exception is CA, which will cause high phytotoxicity in garden cress at concentrations of 3.15 mM and at . The different toxicity of different organic acids was determined by Rao and Mikkelsen (1977), Himanen et al. (2012), and others. The increase in toxicity with increasing acid concentrations was also determined by Camargo et al. (1993), Himanen et al. (2012), and others. Camarago et al. (1993) investigated the toxicity of acetic and butyric acids at concentrations of 0, 0.1, 1,

and 10 mM and found that butyric acid was more toxic than acetic acid because it reduced root growth by 78%, and acetic acid by 55%, which confirms the comparison of butyric and acetic acids in this study. Himanen et al. (2012) investigated the effects of organic acids on germination and seedling growth of garden cress and ryegrass. They also used increasing concentrations of acids (for formic, acetic, propionic, valeric, caproic, and butyric acids, the nominal concentrations were 0.1, 0.3, 0.6, 1.2, 2.4, 4.8, and 9.6 mM) during 72 and 120 h, and found that the toxicity of the tested acids increased with increasing C-chain length, i.e. that butyric acid was more toxic than acetic acid. They also found that the effective concentrations for early seedling growth of garden cress ranged from 0.7 to 2.3 mM and that of ryegrass from 1.2 to 1.8 mM, which is comparable to the values obtained in this study.

The above leads to the conclusion that cucumber is more suitable for the detection of moderate phytotoxicity, and garden cress for the detection of high phytotoxicity of organic acids, that cucumber has greater elasticity in terms of resistance to phytotoxicity by increasing the concentration of organic acids, that cucumber is more sensitive than garden cress to CA and LA with equal sensitivity of cucumber and garden cress to AA and BA. However, cucumber has a greater resistance capacity or resilience than garden cress to increasing concentrations of LA, BA, and AA.

Finally, according to the overall indicators, the least toxic is LA, which at the same time has the most stimulating effect on garden cress. This is followed by CA and AA with slightly higher toxicity than LA, with AA and CA up to medium concentrations having a stimulating effect on garden cress, but not on cucumber roots, while only CA has a stimulating effect on cucumber shoots, not AA. BA is the most toxic, especially for garden cress, on which it has a modest phytostimulating effect at lower concentrations, but has no phytostimulating effect on cucumber even at the lowest concentrations.

CONCLUSION

Garden cress is a more suitable than cucumber for determining the effect of organic acids on germination because organic acids reduced the germination of garden cress more intensely than cucumber. On average, the most intense reduction in GR for both test species was caused by AA. Garden cress was most intensely affected by BA and AA, while only AA had a significant effect on cucumber germination. LA and CA had the weakest effect on garden cress germination, while LA, CA and BA had practically no effect on cucumber germination. Cucumber is more suitable for the detection of moderate phytotoxicity, and garden cress for the detection of high phytotoxicity of organic acids. Also, cucumber is more sensitive than garden cress to CA and LA with equal sensitivity of cucumber and garden cress to AA and BA. Cucumber has a greater resistance capacity or resilience than garden cress to increasing concentrations of LA, BA and AA.

All tested acids had a phytotoxic effect at certain concentrations. The least toxic is LA, including the most stimulating effect on garden cress. Slightly higher toxicity than LA have CA and AA with up to medium concentrations having a stimulating effect on garden cress, but not on cucumber roots. BA is the most toxic, especially for garden cress, on which it has a modest phytostimulating effect at lower concentrations, but has no phytostimulating effect on cucumber even at the lowest concentrations.

The most stimulating effects of organic acids on garden cress roots were at lower or medium concentrations with a similar positive effect on shoots. In cucumber, it is significantly different, as the stimulating effect on roots was completely absent, except for AA with a stimulating effect only at 1.0 mM. A certain stimulating effect on cucumber shoots was found only for AA and CA, but not at the highest concentration.

Simultaneous testing of organic acid toxicity by germination test with garden cress and cucumber is a justified combination due to their different reactions to different types and concentrations of organic acids, and for this reason, such a germination test is recommended for testing the phytotoxicity of all composts and substrates, especially immature composts and those with an acidic reaction.

ACKNOWLEDGMENTS

The paper is the result of research within the project KK.01.1.1.07.0053 *Application of Innovative Bioagents in Sustainable Plant Production Technologies (InoBioTeh)*, funded by the European Union under the Operational Program Competitiveness and Cohesion 2014–2020 from the European Regional Development Fund.

REFERENCES

- Baumgarten, A. & Spiegel, H. (2004). *Phytotoxicity (Plant tolerance)*. Austrian Agency for Health and Food Safety, Vienna, Austria.
- Barral, M. T. & Paradelo, R. (2011). A Review on the Use of Phytotoxicity as a Compost Quality Indicator. *Dynamic Soil, Dynamic Plant*, 5(2), 36–44.
- Brewer, L. J. & Sullivan, D. M. (2003). Maturity and stability evaluation of composted yard trimmings. *Compost Science & Utilization*, 11(2), 96–112.
- Chandrasekaran, S. & Yoshida, T. (1973). Effect of organic acid transformations in submerged soils on growth of the rice plant. *Soil Sci. Plant Nutr.* 19, 39–46.
- Chandra, S., Chauhan, L. K. S., Murthy, R. C., Saxena, P. N., Pande, P. N. & Gupta, S. K. (2005). Comparative biomonitoring of leachates from hazardous solid waste of two industries using *Allium* test. *Science of The Total Environment*, 347(1-3), 46–52. <https://doi.org/10.1016/j.scitotenv.2005.01.002>
- Camargo, F. A. de O., Santos, G. de A. & Rossiello, R. O. P. (1993). Effects of acetic and butyric acids on growth of rice seedlings. *Pesquisa Agropecuaria Brasileira*, 28(9), 1011–1018. <https://doi.org/10.1590/S1678-3921.pab1993.v28.3964>
- EN 16086-2:2012 (2012). Soil Improvers and Growing Media—Determination of Plant Response—Part 2: Petri Dish Test Using Cress. European Standard, Pilsen, Czech Republic.
- Goh, T. B., Huang, P. M. & Rennie, D. A. (1986). Phytotoxicity of organic acids as influenced by montmorillonite, hydroxy-Al montmorillonite and phosphate fertilization. *Communications in Soil Science and Plant Analysis*, 17(5), 515–531. <https://doi.org/10.1080/00103628609367731>
- Gariglio, N. F., Buyatti, M. A., Pilatti, R. A., Gonzalez Rossia, D. E. & Acosta, M. R. (2002). Use of a germination bioassay to test compost maturity of willow (*Salix* sp.) sawdust. *New Zealand Journal of Crop and Horticultural Science*, 30, 135–139.
- Himanen, M., Prochazka, P., Hänninen, K. & Oikari, A. (2012). Phytotoxicity of low-weight carboxylic acids. *Chemosphere*, 88 (4), 426–431.
- Lončarić, Z., Galić, V., Nemet, F., Perić, K., Galić, L., Ragályi, P., Uzingar, N. & Rékási, M. (2024a). The Evaluation of Compost Maturity and Ammonium Toxicity Using Different Plant Species in a Germination Test. *Agronomy* 2024, 14, 2636. <https://doi.org/10.3390/agronomy14112636>
- Lončarić, Z., Novaković, I., Perić, K., Nemet, F., Ravnjak, B., Tkalec Kojić, M., Pravdić, G., Gansberger, M., Uzelac, I., Božić, V. & Vinković, T. (2024b). Bioassay with Four Test Plants for Evaluation of Compost and Vermicompost Suitability as a Growing Medium. *Poljoprivreda / Agriculture*, 30 (2), 25–38. <https://doi.org/10.18047/poljo.30.2.4>
- Manios, V. I., Tsikalas, P. E., Siminis, H. I. & Verdonck, O. (1989). Phytotoxicity of Olive Tree Leaf Compost in Relation to the Organic Acid Concentration. *Biol. Wastes*, 27, 307–317.
- Rao, D. N. & Mikkelsen, D. S. (1977). Effect of acetic, propionic, and butyric acids on rice seedlings growth and nutrition. *Plant and Soil*, 47, 323–334.

15. Rice, E. I. (1984). Allelopathy, 2nd ed. Academic Press, Orlando, FL, USA.
16. Tam, N. F. Y. & Tiquia, S. (1994). Assessing Toxicity of Spent Pig Litter Using a Seed Germination Technique. *Resour. Conserv. Recycl.* 11, 261–274.
17. Tiquia, S. M. & Tam, N. F. Y. (1998). Elimination of Phytotoxicity during Co-Composting of Spent Pig-Manure Sawdust Litter and Pig Sludge. *Bioresour. Technol.* 65, 43–49.
18. Ulbright, C. E., Pickard, B. G. & Varner, J. E. (1982). Effects of short chain fatty acids on radicle emergence and root growth in lettuce. *Plant Cell Environ.*, 5, 293–302.
19. Wong, M. H. (1985). Phytotoxicity of Refuse Compost During the Process of Maturation. *Environ. Pollut.*, 37, 159–174.
20. Zucconi, F., Monaco, A., Forte, M. & de Bertoldi, M. (1985). *Phytotoxins during the stabilization of organic matter. Composting of Agricultural and other Wastes.* Elsevier Applied Science Publishers, Barking, UK.
21. Zucconi, F., Pera, A., Forte, M. & de Bertoldi, M. (1981a). Evaluating toxicity of immature compost. *BioCycle*, 22, 54–57.
22. Zucconi, F., Forte, M., Monaco, A. & de Bertoldi, M. (1981b). Biological Evaluation of Compost Maturity. *BioCycle*, 22, 27–29.

OCJENA FITOTOKSIČNOSTI ORGANSKIH KISELINA TESTOM KLJAVOSTI

SAŽETAK

Fitotoksičnost, bez obzira na uzrok, može značajno smanjiti rast i razvoj biljaka od klijanja sjemena nadalje. Stoga je test klijanja najčešće korištena laboratorijska metoda za određivanje fitotoksičnosti, a kres salata i krastavac najčešće se koriste kao testne biljke. Cilj ovoga istraživanja bio je utvrditi fitotoksični i/ili fitostimulacijski učinak različitih koncentracija octene (AA), mliječne (LA), maslačne (BA) i limunske (CA) kiseline. U testu klijanja, sjeme kres salate i krastavca izloženo je različitim koncentracijama (0, 0,1, 0,25, 0,5, 1,0, 2,0, 4,0 i 8,0 mM) gore navedenih organskih kiselina kako bi se odredile i usporedile koncentracije s fitotoksičnim učinkom. Rezultati pokazuju da je kres salata prikladnija za određivanje učinka na klijanje i učinka visoke fitotoksičnosti, a krastavac za određivanje umjerene fitotoksičnosti. Sve testirane kiseline imale su fitotoksični učinak pri određenim koncentracijama. Najmanje toksična bila je LA, nešto toksičnije bile su CA i AA, a BA je bila najtoksičnija, posebno za kres salatu. Najstimulativniji učinci na kres salatu bili su pri nižim ili srednjim koncentracijama, a stimulirajućega učinka na krastavac gotovo uopće nije bilo. Test kljavosti s kres salatam i krastavcem opravdana je kombinacija zbog njihovih različitih reakcija na organske kiseline te se preporučuje za ispitivanje fitotoksičnosti svih komposta i supstrata, posebno nezrelih kiselih komposta.

Ključne riječi: test kljavosti, fitotoksičnost, indeks kljavosti, octena kiselina, mliječna kiselina, maslačna kiselina, limunska kiselina

(Received on April 20, 2026; accepted on May 25, 2026 – Primljeno 20. travnja 2026.; prihvaćeno 25. svibnja 2026.)