Date: April-May 2019

Location: Minoprio Analisi e Certifications, Italy

Commissioned by: Biomedic Clinic and Research

Crops: Spring Barley

Summary of Toxicity Trial

After careful study, the researchers found that using the Harvest Harmonics micro-transmitters system did not inhibit germination or growth of the plants. The research facility concluded that the differences in germination and growth of the plants was statistically insignificant, thus proving that the Harvest Harmonics system produced no phytotoxic effects on the treated plants.

For full details, please see the complete report attached.

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Subject: phytotoxicity test results of the technological application of the irrigation water activation - ref. MAC Offers 48/2019 + 93/2019

PREMISE

In relation to the offers indicated in the subject, an assignment was conferred to Minoprio Analisi e Certifications S.r.l. di Fondazione Minoprio (CO) to carry out preliminary experimental activities in order to verify the possible effects on the vegetative activity of a technological application of irrigation water activation implemented by you.

The experimental test carried out involved the implementation of a phytotoxicity test to assess any negative effects of water treatment.

The results of the present preliminary experimentation may lead to the definition of tests on a larger scale.

The experimental test was carried out at the Minoprio Foundation facilities.

The experimental methodology implemented, results and conclusions are shown below.

MATERIALS AND METHODS

The job order included the realization of the following experimental test.

Spring barley test (ref. UNI EN 1608601_2012)

The test aims to assess any phytotoxic aspects on germination / growth of plant species (germination and growth inhibition).

As a substrate, according to the reference standard, peat was used with a humidification degree of H3-H5 (Van Post scale), with addition of calcium carbonate for pH correction and 2.25 g of 18-11-18 fertilizer (N - P_2O_5 - K_2O).

Spring barley was used for the test.

Sowing took place on 03/26/2019 in pots with a capacity of 750 ml (20 seeds / pot).

The pots were placed in an iron / glass greenhouse (Hortiplus glasses), on pallets with ducts, with a minimum temperature of 12° C and aeration at 18° C.

The pots were divided into two experimental theses:

- *Specimen 1 (control): irrigation with tap water;*
- *Specimen 2 (test): irrigation with activated water.*

For each specimen, 4 replicas have been prepared, with 3 replica pots (total 240 seeds / specimen).

For the first 5 days pots were covered with non-woven fabric and kept moist with nebulization* (always differentiating the two types of irrigation water); on the fifth day, the germination rate for each pot was determined.

Subsequently the irrigation was carried on at first with a nebulizer and then with a watering can or a spray gun, always with the utmost care to avoid contamination between the plants of the different experimental theses.

On 04/23/2019 the fresh and dry (75° C) biomasses were determined for each individual pot (replicas 2, 3, 4).

* nebulization: watering via a mist or microjet spray

The results were subjected to statistical analysis to verify the possible presence of germination and growth inhibition.

Replica 1 pots were left in cultivation without irrigation until May 6th , 2019, date on which the irrigation with the two different types of water was restored.

The observation of any signs of recovery was kept active until May 15th , 2019.

On May 10th, on samples of treated and untreated water, the main irrigation water characterizing parameters were determined.

RESULTS AND STATISTICAL INTERPRETATION

The following tables summarize the results obtained and the outcome of the processing of the same (analysis of variance and Duncan test: different letters correspond to significantly different values for P = 0.05).

Spring barley test (ref. UNI EN 1608601_2012)

The results obtained highlight a significant absence of germination and growth inhibition.

Table 1 shows the results related to the germination rate. Data shows a significant results homogeneity (modest coefficient of variation) and a complete absence of germination inhibition using activated irrigation water (specimen 2).

Table 1: germination inhibition

thesis	replica	pot	germinated seeds number	germination rate (%)	germination average rate (%)	germination rate coefficient of variance	germination inhibition (%)
1 - control	1	1	20	100			
1 - control	1	2	18	90			
1 - control	1	3	20	100			
1 - control	2	1	18	90			
1 - control	2	2	18	90			
1 - control	2	3	18	90			
1 - control	3	1	20	100	92.08	15.79	=
1 - control	3	2	17	85			
1 - control	3	3	17	85			
1 - control	4	1	17	85			
1 - control	4	2	18	90			
1 - control	4	3	20	100			
2 - treated	1	1	20	100			
2 - treated	1	2	20	100			
2 - treated	1	3	18	90			
2 - treated	2	1	17	85			
2 - treated	2	2	20	100			
2 - treated	2	3	19	95			
2 - treated	3	1	18	90	92.08	19.17	**0.00**
2 - treated	3	2	20	100			
2 - treated	3	3	18	90			
2 - treated	4	1	16	80			
2 - treated	4	2	16	80			
2 - treated	4	3	19	95			

Growth outcomes are shown in Table 2 (fresh aerial biomass weight) and in Table 3 (dry aerial biomass weight). Assuming that the reference standard indicates to quantify the growth inhibition on the fresh biomass weight only, the test conducted has provided the calculation also on the dry weight, considering this data more robust and reliable.

Table 2: growth inhibition (fresh weight)

thesis	replica	pot	avg fresh wt (g)	avg fresh wt / pot (g)	plants nos. / pot	plant fresh wt pot (g)	plant avg fresh wt pot (g)	plant wt coef. of variance	germination inhibition (%)
1 - control	2	1	22.26		19	1.17			
1 - control	2	2	20.02		19	1.05			
1 - control	2	3	15.78		19	0.83			
1 - control	3	1	20.61		19	1.08			
1 - control	3	2	18.07	19.124	16	1.13	1.019	36.74	=
1 - control	3	3	26.53		20	1.33			
1 - control	4	1	13.67		19	0.72			
1 - control	4	2	17.40		18	0.97			
1 - control	4	3	17.78		20	0.89			
2 - treated	2	1	15.07		20	0.75			
2 - treated	2	2	20.90		19	1.10			
2 - treated	2	3	16.50		19	0.87			
2 - treated	3	1	19.17		20	0.96			
2 - treated	3	2	13.89	17.088	17	0.82	0.938	23.72	**10.65**
2 - treated	3	3	18.29		18	1.02			
2 - treated	4	1	16.23		17	0.95			
2 - treated	4	2	19.91		19	1.05			
2 - treated	4	3	13.83		15	0.92			

Table 3: growth inhibition (dry weight)

thesis	replica	pot	avg dry wt (g)	avg dry wt / pot (g)	plants nos. / pot	plant dry wt pot (g)	plant avg dry wt pot (g)	plant wt coef. of variance	germination inhibition (%)
1 - check	2	1	2.00		19	0.105			
1 - check	2	2	1.83		19	0.096			
1 - check	2	3	1.38		19	0.073			
1 - check	3	1	1.84		19	0.097			
1 - check	3	2	1.55	1.683	16	0.097	0.090	34.64	=
1 - check	3	3	2.19		20	0.110			
1 - check	4	1	1.18		19	0.062			
1 - check	4	2	1.58		18	0.088			
1 - check	4	3	1.60		20	0.080			
2 - treated	2	1	1.33		20	0.067			
2 - treated	2	2	1.82		19	0.096			
2 - treated	2	3	1.41		19	0.074			
2 - treated	3	1	1.69		20	0.085			
2 - treated	3	2	1.17	1.482	17	0.069	0.081	26.23	**11.95**
2 - treated	3	3	1.63		18	0.091			
2 - treated	4	1	1.36		17	0.080			
2 - treated	4	2	1.78		19	0.094			
2 - treated	4	3	1.15		15	0.077			

The results show a modest growth inhibition (10.65% for fresh weight and 11.95% for dry weight). It can be stated with certainty that this value is not statistically significant, meaning that the use of activated water has no phytotoxic effects on plant growth.

This statement is confirmed by the statistical analysis (ANOVA) carried out on the results obtained (Table 4) and the Duncan test results (95% confidence).

Table 4: Duncan test results with 95% confidence

Thesis	Count	**Average fresh g**	Standard deviation	Coefficient of variance	Homogeneous groups
1 - control	9	**19.1244**	3.78339	19.783%	**a**
2 - treated	9	**17.0878**	2.60458	15.2423%	**a**
Thesis	Count	**Average dry g**	Standard deviation	Coefficient of variance	Homogeneous groups
1 - control	9	**1.68333**	0.31301	18.5946%	**a**
2 - Treated	9	**1.48222**	0.254695	17.1833%	**a**

(if present, statistically different averages for P = 0.05 correspond to different letters)

The results of the water analysis carried out on May 10th highlighted the absence of significant differences for the main characterization parameters for irrigation water (pH, electrical conductivity, carbonates and bicarbonates, calcium, magnesium, sodium, SAR).

The results are reported in the attached Test Reports no. 19211/564 and 19211/565 issued on May 13th 2019.

COMMENT ON RESULTS AND FINAL CONSIDERATIONS

The results of the growth test with spring barley showed no phytotoxicity of the water treatment system.

Available for clarifications and further details.

for Minoprio Analysis and Certifications

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(with the collaboration of Dr. Piero Frangi of the Minoprio Foundation)

Vertemate con Minoprio, June 10th, 2019