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Original article

Effect of extracts of humic substances on germination and growth of rice plant (*Oryza sativa* L), *cv*. INCA LP-5.

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ABSTRACT

The use of biostimulants in agriculture is gaining ground in the international context with very promising results for the agriculture sustainability in the new millennium. The objective of the present work was to evaluate the biological activity of humic substances extracts (HSE I) and (HSE II) in the rice crop *cv* INCA LP-5. An experiment was carried out in the germinative phase: rice seeds were germinated in dilutions of the HSE I and HSE II extracts and the percentage of germination and root and coleoptile length were evaluated 7 days after germination seed (dag). Another in the vegetative phase: rice seeds were imbibed 24 hours in dilutions of HSE I and HSE II, then sowed and were applied by foliar spray dilutions of HSE I and HSE II at 7 and 14 dag, the indicators of plant height and root length were determined at 14 and 21 dag. The extracts of humid substances used have no effect on the percentage of germination of the rice seeds, however the use of dilutions of HSE II promoting the length of the root and coleoptile of the rice seedlings. The effect of foliar application of both HSE was perceptible in the aerial part; in the root only the use of I HSE in the 1:60 dilution was beneficial. Other study will be necessary for the use of I HSE and II in rice cultivation.

Key words: biostimulants, cereals, foliar application, vermicomposts

INTRODUCTION

The biostimulants used in agriculture, which include humic substances (HS), increase the efficiency in nutrition, tolerance to abiotic stress and the quality of the crops [(1,2)]. HS are formed by chemical and biological transformations of plant and animal material, as well as microbial metabolism, and represent the largest carbon reserve on the earth's surface [(3)]. These substances are defined chemically as a mixture of various small molecules that form a supramolecular association stabilized by hydrophobic interactions and hydrogen bonds [(4)] and they are classified according to their solubility as a function of pH in humic acids (HA) and fulvic acids (FA).

The effects of HS on plants are complex and involve non-linear and interrelated dynamic processes that must be treated with an interdisciplinary vision: architecture of the roots, membrane H^+-ATPase activity, primary and secondary metabolism, proteins and gene expression, those that are dependent on environmental conditions, plant type and their ontogenesis [(5)]. The intensity and HS bioactivity type in plants is more related to their structural complexity (conformation and structural organization) than to structure type present in them [(6)].

Most of the HS used in agriculture are currently derived from non-renewable sources and the humic product development from renewable sources is required [(3)], as it is the case of HS isolated from vermicompost. In the Chemistry Department of the Agronomy Faculty from Agrarian University of Havana, for several years different liquid products (HS: HSE extracts) and solid (solid waste obtained from the HS: RS extraction process) have been developed based on humic substances extracted from bovine manure vermicompost. In other works [(7,8)] the results of several studies with HSE and RS in agriculture and the environment are compiled, and it is pointed out that these products can increase agricultural production, improve soil conditions and retain cations of heavy metals present in liquid waste. Several studies show that the foliar application of HSE in different crops generates beneficial effects on their biological and agricultural productivity [(9-12)]. In rice cultivation, studies of the protective effect of HA isolated from vermicompost against oxidative stress and hydric stress are reported [(13-15)], however studies in this crop on the use of HSE are scarce. In this way, this work aimed to evaluate the biological activity of two extracts of humic substances (I and II HSE) in the cultivation of rice (*Oryza sativa* L.), cultivar INCA LP-5.

MATERIALS AND METHODS

The study was developed in the laboratories of the Department of Chemistry of the Agronomy Faculty at the Agrarian University of Havana (UNAH), during the first semester of 2015. The short-cycle INCA LP-5 cultivar [(16)] using the hybridization method of the varieties CP1C8 and 2077, which was

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donated and certified by the Base Scientific Technological Unit (UCTB) Los Palacios, Pinar del Río, belonging to the National Institute of Agricultural Sciences (INCA).

The humic substance extracts of (I and II HSE) were obtained from a bovine manure vermicompost supplied by the Guayabal farm belonging to the UNAH Scientific Complex, which was carried out in piles on soil with African red worms (*Eiseinafoetida*) during three months. The sifted (2 µm) and dry bovine manure vermicompost was subjected to extraction with a basic solution ($KOH/Urea/KH_2PO_4$- 0.15/0.1/0.03 mol L^{-1}), in a ratio of 1: 10 (m: v), with modifications [17] and methodology proposed by Lukambani [18]. In Figure 1 the flow diagram for obtaining HSE is presented, some chemical and physicochemical properties of the extracts obtained are reported in Table 1.

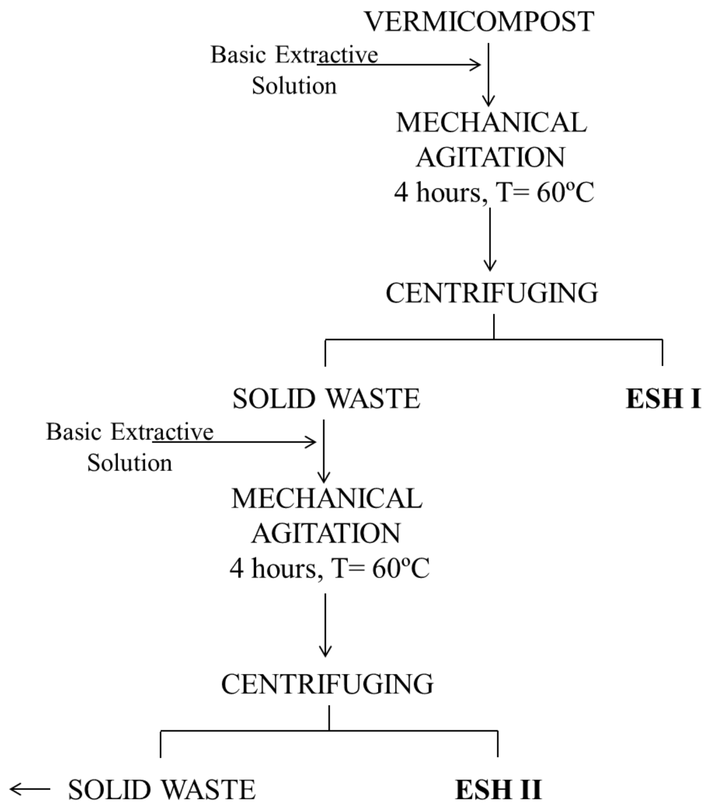


Figure 1. Protocol for obtaining extracts of humic substances HSE I and HSE II.

Table 1. Chemical and physicochemical properties of extracts of humic substances HSE I and HSE II according to Lukambani

Properties	HSE I	HSE II
pH	7.33 ± 0.01	7.09 ± 0.01
TDS ($g.L^{-1}$)	5.51± 0.01	5.04 ± 0.01
C.E ($mS.cm^{-1}$)	9.53 ± 0.01	8.67 ± 0.01
% Salts	0.52 ± 0.01	0.47 ± 0.01
% Organic C	21.30 ± 1.25	28.90 ± 1.22

The biological activity of extracts of humic substances in rice cultivation was evaluated in the germination phase and in the vegetative one, setting up an experiment for each of these. In the germination phase, rice seeds previously selected by flotation were soaked in water for 24 h; after this time, they were air-dried and placed to germinate in a petri dish with dilutions of the HSE I and HSE II extracts (Table 2), at a rate of 100 seeds per plate. At 3, 5 and 7 days after being placed to germinate, the germinated seeds were counted, taking the radicle emission as germination criteria. The germination percentage was calculated and radicle lengths (in cm) and of the coleoptile (in cm) were measured at 15 seedlings per treatment, corresponding to 5 seedlings per plate.

Table 2. Treatments used to evaluate the effect of different dilutions of the HSEs in the INCA LP-5 cultivar

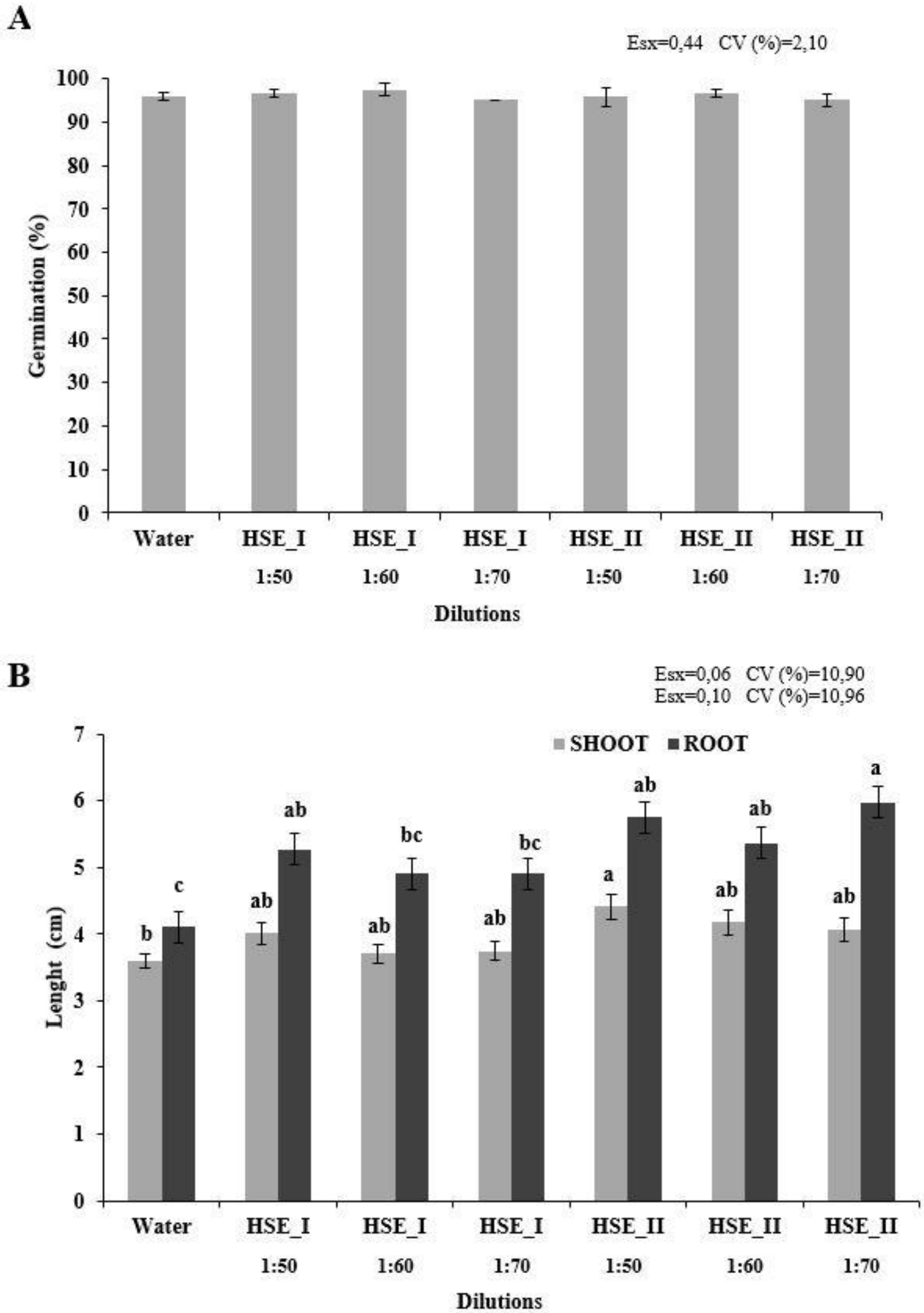
Treatments		Dilutions of HSE (v:v)
T1	Water	-
T2	HSE _I	1:50
T3	HSE _I	1:60
T4	HSE _I	1:70
T5	HSE _II	1:50
T6	HSE _II	1:60
T7	HSE _II	1:70

For the vegetative phase, the previously selected seeds were soaked for 24 h in water and in the different dilutions of HSE I and II HSE, (Table 2). Subsequently, approximately 50 seeds were sown in plastic trays (18x3 cm) with Gleysol Vertic soil [(19)]. At 7 and 14 days after germination the foliar spraying (200 mL) of HSE I and HSE II dilutions was carried out, maintaining the treatments described previously in Table 2. At 14 and 21 days after germination; 15 seedlings were randomly sampled per treatment distributed in 5 seedlings per tray, and the height of the seedling (cm) and root length (cm) were measured.

In both experiments, a completely randomized design was used with 7 treatments with 3 replications for each one. The data were processed through a Simple Classification Analysis of Variance (ANOVA) with the Statgraphics Plus software version 5.1, using the arcs √ (%) transformation when required and when significant differences were found between the means, the Tukey test was applied at 95 % probability.

RESULTS AND DISCUSSION

In the germination phase, the desired physiological effect on germination is seen for all treatments (Figure 2A) (more than 90 % of the seeds germinate), in addition that the dilutions used of the humic extracts HSE I and HSE II do not affect the percentage germination of rice seeds.



a b unequal letters show statistical differences according to Tukey at 95 %

Figure 2. Germination percentage (A), coleoptile and radicle length (B) of rice seedlings treated with two extracts of humic substances 7 days after germination

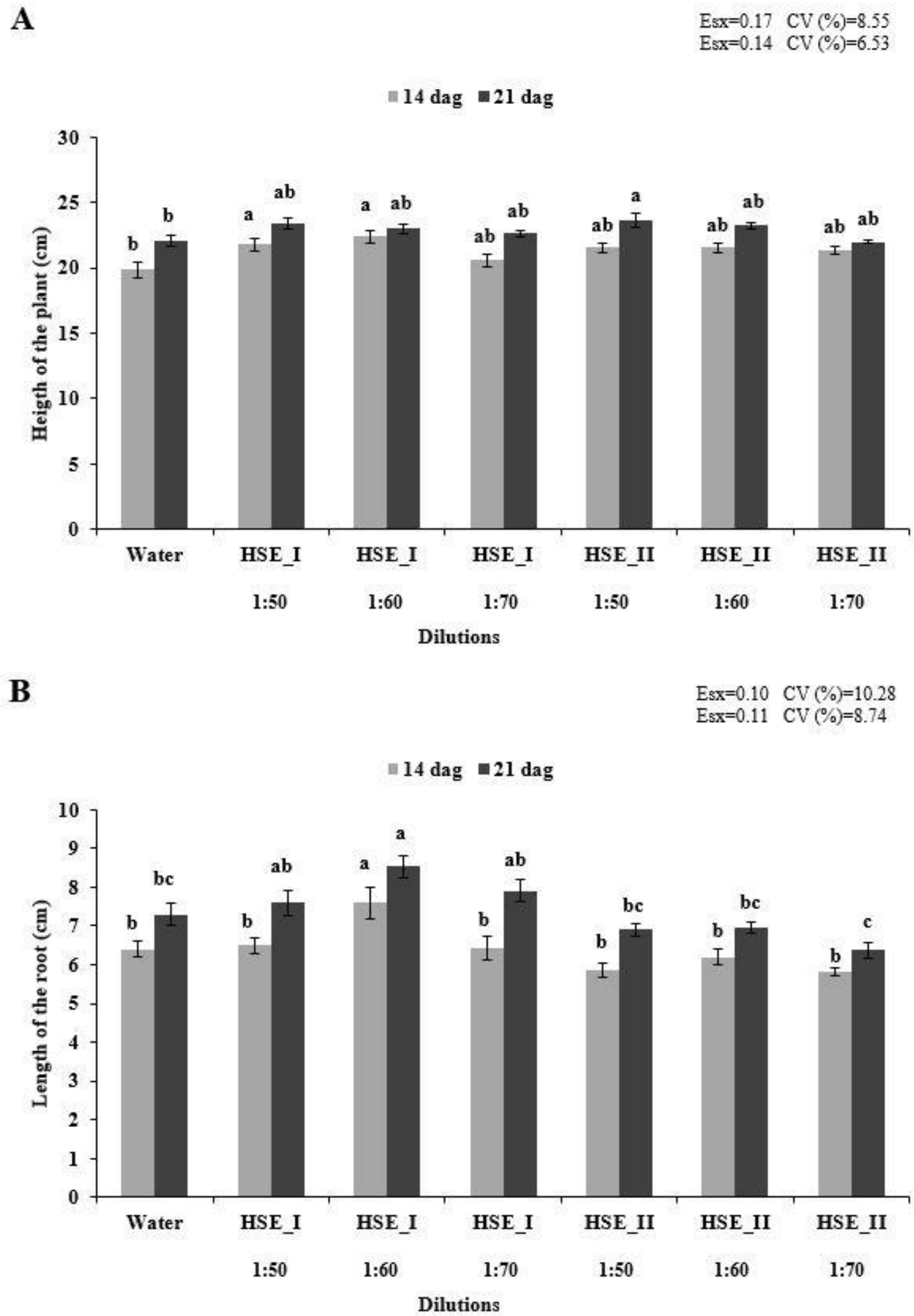
There are few reports of HS effect on the germination percentage, a process in which complex metabolic and cellular events occur, which begins with the water absorption and ends with the radicle emergence and does not include seedling growth [(20)].

The indicators coleoptile length and radicle length of rice seedlings at 7 dag the seeds (Figure 2B) are modified with the use of humic substance extracts HSE I and HSE II. A tendency to increase the coleoptile length with HSE use is appreciable, however only the T5 treatment, which corresponds to

HSE II (1:50), differs significantly from the control treatment (T1). In the radicle length case, with the exception of treatments T3 and T4, the significant increase of this indicator with the HSE use is perceptible. However, only the T7 treatment, which corresponds to HSE II (1:70), differs significantly and positively from the control treatment (T1).

The HS stimulating action on seedling growth has been reported by several authors (3,7,21,22). It has been associated with an increase in the activity of the plasma membrane H+-ATPase enzyme (23-25) in the same way that exogenous auxins induce plant growth also the presence of different phytohormones in HS structure (26). Besides, the production of reactive oxygen species (ROS), which function as signal molecules, causing events such as membrane hyperpolarization and the activation of intra and extracellular Ca^{2+} channels (27). Furthermore, the recalcitrance and lability of humic fractions are chemical properties that define the stimulation of plant root parameters, where root length and the emission of smaller roots are related to less complex structures and functionalized (6).

The results of HSE foliar application in rice vegetative phase are presented in Figure 3 (A and B). The rice plant height (Figure 3A), in both evaluation moments, is generally stimulated with the HSE use. While at 14 dag treatments T2 and T3 (HSE I dilution 1:50 and 1:60 respectively) show significantly higher height values than the control, at 21 dag only treatment T5 (HSE II dilution 1:50) is the one that shows this behavior.



a b unequal letters show statistical differences according to Tukey at 95 %

Figure 3. Plant height (A) and root length (B) of rice seedlings treated with two humic substance extracts at 14 dag and 21 dag

The same behavior was not observed in the root length indicator (Figure 3B). At both 14 dag and 21 dag, only the T3 treatment showed significantly higher values than the control. Furthermore, at 21 dag, although an inhibitory effect of the use of HSE II is not observed, it is observed that this extract dilution use resulted in the lowest values of root length. It is possible that with the HSE II extract the use of other dilutions is necessary, considering that the organic C content of this extract is higher than that of HSE I (Table 1).

The plant growth stimulation mechanisms by HS foliar application are few discussed. The main investigations are related to these substance application by the root; however, the stimulating effect of foliar application is well documented. In a review on the use of HA and FA as biostimulants in horticultural plants [3]. Several works are reported where HS foliar application is carried out and the effects found are of increases in flowering, fruiting, production, fruit quality and efficient use of nutrients, few studies (3 of 19 studies reported with foliar application) report the effect on root growth.

The HSE of this work are potassium humates isolated from bovine manure vermicompost, obtained from the protocol proposed [18], who modified the Hernández protocol [17], so that our results correspond to those reported by some authors [28]. They, in the evaluation of the foliar application of dilutions of a humate isolated from vermicompost in the lettuce crop, found that the 1:60 dilution produced the most notable impacts on the indicators of biological and agricultural productivity, reducing the crop's production cycle by 21 days, which greatly benefits the producers of this vegetable.

It is possible that the stimulating action observed for HSE is due to the HS they contain (HA and FA). Its polyfunctionality and heterogeneous structural composition may contain compounds that stimulate enzymatic activities [28] or to the presence of other components such as amino acids, minerals, phytohormones or precursors of phytohormones, as well as microorganisms [8, 17,18], or perhaps the joint action of these.

The results found in this work suggest the need to evaluate in future research, the structural differences that the humic substances of each of the extracts could present. Also, the doses and forms of application of the HSE, including the possibility of combining them with plant growth promoting bacteria [29] so that these biostimulants can be included in the organic and sustainable production of rice.

CONCLUSIONS

- The HSE use does not modify seed germination percentage, but benefits rice seedling growth, especially with HSE II dilution use.
- The HSE foliar spraying benefits the growth of the plant aerial part, the effect on root growth is only observed with HSE I use 1:60 (v: v).

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