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ANTI-STRESS EFFECTS OF HUMIC ACIDS OF VERMICOMPOST IN TWO RICE (*Oryza sativa* L.) CULTIVARS

Efectos anti estrés de ácidos húmicos de vermicompost en dos cultivares de arroz (*Oryza sativa* L.)

Reinier Hernández[1]✉, Celerino Robles[1], Andrés Calderín[2], Fernando Guridi[3], Inés M. Reynaldo[4] and Deborah González[4]

ABSTRACT. Humic acids (AH) were extracted and isolated from bovine manure vermicompost, and they were partially characterized using UV-vis and / FT-IR. They were sprinkled foliarly at 32 days after germination coinciding with the start-up phase tillage in rice cultivation (*Oryza sativa* L.) under conditions of irrigation and water deficit. Three concentrations of AH (30, 34 and 38 mg L^{-1}) were used, in addition to a control treatment without application. Specifically in the active tillering stage, the effect of foliar application of AH under irrigation conditions and water deficit was evaluated in indicators such as: plant height, dry mass in the root section, peroxidase enzyme activity (POX) and content of soluble proteins. The results indicated positive effects of the AH on the biochemical - physiological indicators evaluated in the vegetative phase, with effects on the dry mass in the radical part up to 40 % higher than the control, observing evidence of a possible protective effect against the water deficit by the AH. It was concluded that a possible response could be the mimetic action of AH with the abscisic acid hormone (ABA), producing equivalent effects to this.

Key words: hydric stress, peroxidase, total proteins, humic substances

RESUMEN. Los ácidos húmicos (AH) de vermicompost fueron extraídos, aislados y caracterizados parcialmente usando UV-vis y /FT-IR. Los mismos fueron asperjados foliarmente a los 32 días después de germinado coincidiendo con la fase de inicio del ahijamiento en el cultivo del arroz (*Oryza sativa*. L), en los cultivares J-104 e IACuba-33, bajo condiciones de aniego y déficit hídrico. Tres concentraciones de AH (30, 34 y 38 mg L^{-1}) fueron utilizadas, además de un tratamiento control sin aplicación. Específicamente en la etapa de ahijamiento activo, se evaluó el efecto de la aplicación foliar de los AH bajo condiciones de aniego y déficit hídrico en indicadores como: altura de la planta, masa seca en la parte radical, actividad de las enzimas peroxidasas (POX y contenido de proteínas solubles. Los resultados indicaron efectos positivos de los AH sobre los indicadores bioquímico - fisiológicos evaluados en la fase vegetativa, con efectos sobre la masa seca en la parte radical de hasta 46 % superior al control, observándose evidencias de un posible efecto protector ante el déficit de agua por parte de los AH. Se llegó a la conclusión que una posible respuesta pudiera ser la acción mimética de los AH con la hormona ácido abscísico (ABA), produciendo efectos equivalentes a esta.

Palabras clave: estrés hídrico, peroxidasas, proteínas totales, sustancias húmicas

INTRODUCTION

The rice is a cereal cultivated in all the continents, being Asia the one of greater production and consumption. This crop requires an adequate management of the water resource, which is why in most cases it is cultivated under flooding conditions (1). In Cuba, rice has been cultivated since colonial times. From 1967 on, it became very important in the economic plans of the country. The importance of rice as food for Cuban society can be understood from the high per capita annual consumption, estimated

[1] Instituto Politécnico Nacional-CIIDIR-Unidad Oaxaca, Laboratorio de Suelos, Calle Hornos 1003, 71230 Santa Cruz Xoxocotlán, Oaxaca, México
[2] Universidad Federal Rural de Rio de Janeiro (UFRRJ). BR 465 km 7. CEP 23890-000, Seropédica, RJ. Brasil
[3] Universidad Agraria de La Habana (UNAH). Autopista Nacional Km 23 ½ y Carretera de Tapaste. San José de las Lajas. Mayabeque
[4] Instituto Nacional de Ciencias Agrícolas (INCA), Carretera Tapaste, Km 3½, Gaveta Postal 1. San José de las Lajas, Mayabeque. Cuba. CP 32700
✉ reinierhc86@hotmail.com

at around 60 kg, above almost all the countries of the American continent. Currently there are different productive forms in Cuba that contribute to the national production of rice UBPC (Basic Unit of Corporate Production), CCS (Credit and Service Cooperative), CPA (Agricultural Production Cooperative), UEB (Business Unit of Base) and private producers (2). The average national production in Cuba has never exceeded 3,600 kg ha^{-1} of paddy rice, despite the great technological efforts that the country invests. One of the causes that influence these low yields is the instability in the supply of irrigation water (3). This shortage of water brings with it the induction of water deficit in the plants, due to a decrease in its availability in the soil (4). The water deficit is the abiotic stress of greater incidence in the growth and development of the plants, and specifically one of the limiting factors in the productivity of the rice (5).

The use of humic substances (specifically the AH) is emerging as a technological option to mitigate the damage caused by water deficit, induce greater resistance to crops and increase rice production. Evidence has been published of the AH potentialities to exert positive effects on the different properties of the soil-plant system (6) and present similar effects to natural hormones under water stress conditions stimulating the synthesis of Peroxidases (POXs) and Catalases (CAT), with the objective of converting reactive oxygen species (ERO) into harmless species for plants and reducing oxidative stress caused by water deficit (7-9). However, the works that inform studies on the role that these could play as protectors against different types of stress are not yet enough.

The objective of this investigation was to evaluate the effect of the application of AH extracted from vermicompost in the stage of tillering of the rice cultivation (*Oryza sativa* L.) of IACuba-33 and Jucarito-104 (J-104) cultivars, under conditions of flooding and water deficit.

MATERIALS AND METHODS

The experimental activities were carried out in the Chemistry Department of Agronomy Faculty - Agrarian University of Havana (UNAH), in coordination with the National Institute of Agricultural Sciences (INCA), Department of Plant Physiology and Biochemistry, where the enzymatic analyses were done. The extraction procedure of the AH was carried out in the laboratories of the Department of Chemistry (UNAH) and in the Plant Physiology Laboratory (INCA). The AH were characterized in the Soil Department of the Agronomy Institute of the Federal Rural University of Rio de Janeiro, Brazil (10).

The bovine manure vermicompost used came from the "El Guayabal" farm, located in the San José de las Lajas municipality, Mayabeque province, Cuba, with coordinates 22°59'55.95'' North Latitude and 82°10'10, 27" West Length.

The evaluations of the plants growing under semicontrolled conditions were made in the phenophase of active tillering. These were developed in the area of acclimatization of the Plant Biotechnology group of the Department of Biology-Health of the UNAH. A completely randomized design was used, with a complete trifactorial experiment factor 1: AH concentrations, with four levels (0, 30, 34 and 38 mg L^{-1}); factor 2: rice cultivars, with two levels (Jucarito104 and IACuba-33); factor 3: crop water condition, with two levels (with and without water stress). The resulting 16 treatments were repeated three times, for a total of 48 experimental units. Each experimental unit was a tray with dimensions: 45 cm long, 30 cm wide and a depth of 20 cm. The soil used was Gley Vertex chromic nodular ferruginous, placing 6.5 kg in each tray (11). The solutions of AH were sprayed foliarly at a rate of 3 mL per plant, on only one occasion, five days before the registration of data of the variables evaluated. For the management of the water in the conditions of watering in the cultivars used, the recommendations of the Technical Rice Instruction were followed (1).

In the case of soil water deficit treatments, water management was carried out as follows: after five days after germination had occurred, the first irrigation was carried out; later it was watered with a daily frequency during three days, being carried out the thinning to the fourth day, leaving 10 plants in each tray. The daily irrigations continued until the crop reached 15 days before the active tillering, at which time the irrigation was suspended, with the aim of inducing water deficit in this stage (12).

The cultivar Jucarito-104 of medium cycle, recommended for flooding conditions (13), was donated by the Scientific Technological Unit of Los Palacios Base, Pinar del Río (UCTB) belonging to the INCA; and the IACuba-33 short-cycle cultivar, recommended for conditions of low water input and nitrogen fertilizers, was donated by the Grain Research Institute. The concentrations of AH used were 30, 34 and 38 mg L^{-1} (14). The indicators evaluated were: growth in height (measured from the soil surface to the top of the highest leaf), using a Lugarex 22514 millimeter ruler with a margin of error of 0.1 cm; dry mass of the radical part, dried in an oven (model Venticell Medcenter) at 80 °C until constant weight, then weighed using an analytical balance (Sartorius model, precision ± 0.0001 g).

The enzymatic activity of peroxidases POXs (15) and the content of total soluble proteins (16) were determined using a total of five plants per treatment, from which three samples of 0.25 g of leaf tissue were extracted.

Prior to the statistical analysis, normality of the data and homogeneity of variances were verified. The statistical analyzes performed were a simple ANOVA and separation of means by Tukey's DSH. The statistical program STATGRAPHICS version 5.1 was used. All the initial data were processed by the Microsoft Office 2010 Excel tool.

RESULTS AND DISCUSSION

Chemical characterization of the extracted AH

Uv-Vis spectroscopy, elemental composition and content of functional groups

The UV-Vis absorption spectrum of the AH shows the presence of an intense band at the shortest wavelengths, which oscillate between 200-250 nm, which is assigned to structures with unsaturated bonds (17). The E_4/E_6 ratio was 4.22, the range of values published for AH is 3 to 5 (18) (Figure 1).

The values indicated in Table 1 show that the AHs obtained have a composition that is within the ranges reported for that type of humic substance extracted both for composted and vermicomposted materials (19), and those present in soils (20).

The H/C ratio has a similar value to the results already published in other investigations (10). Its low value is an indication of the high degree of aromaticity present in the structure, which reaffirms what is found in UV-Vis spectroscopy and the E_4/E_6 ratio. The value of the O/C ratio is considered high, indicative of an extensive presence of oxygenated structures (hydroxides, carboxyl, phenols and quinones). The value of the C/N ratio is similar to published values for this type of substance.

Figure 2 shows the FTIR spectrum of the AH, which showed similarity with those reported in the literature for composted and vermicomposted materials (7).

Figure 3 shows the results of the leaf sprinkling of the AH in the height variable of the plant in the cultivars J 104 and IACuba-33 in semi-controlled conditions, under flooding and water deficit of the soil.

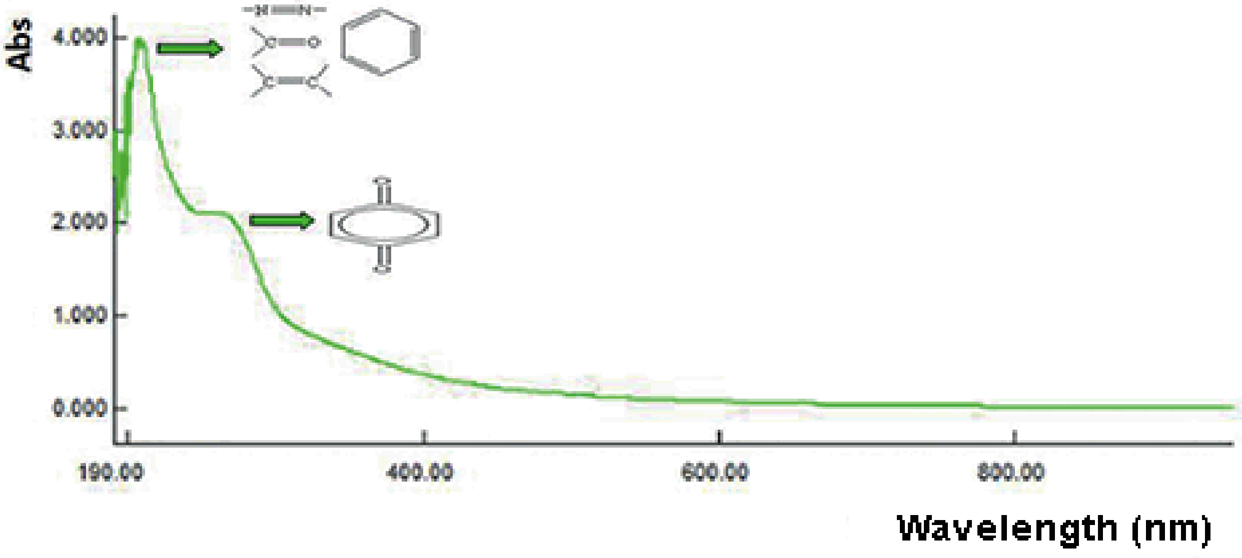

Figure 1. Ultraviolet-visible spectrum (UV-Vis) of humic acids (AH) extracted from vermicompost of bovine manure

Table 1. Elemental composition, percentages of Carbon (C), Hydrogen (H), Nitrogen (N) and Oxygen (O) and the relationships between carbon and other elements, in the AH extracted from vermicompost of bovine manure (means of three repetitions)

C	H	O	N	S	H/C	O/C	C/N	Total Acidity	Carbonyl	Phenols	E4/E6
% (m:m)								mol kg^{-1} (C)			
56,7	4,84	34,6	3,07	0,7	0,08	0,61	18,4	9,24	2,03	11,27	4,22

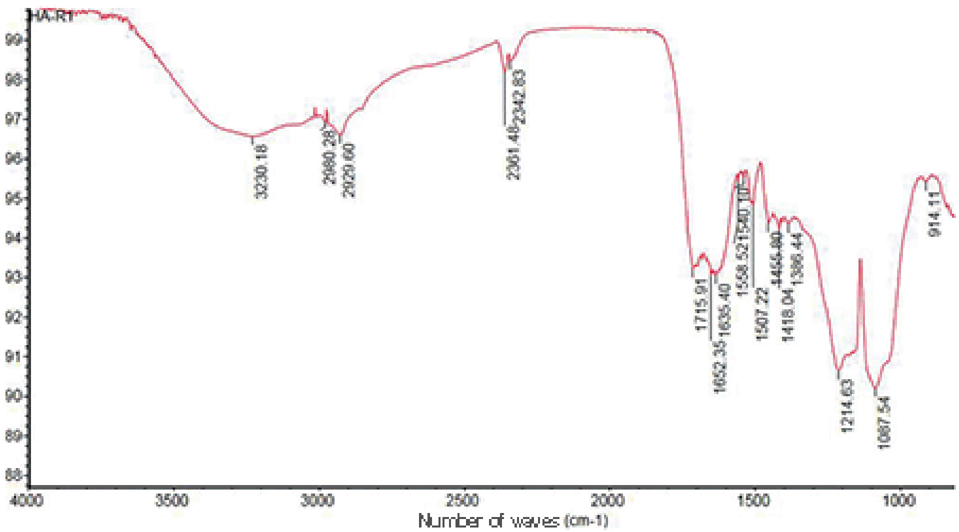

Figure 2. Fourier transform infrared (FTIR) spectrum of humic acids (AH) extracted from vermicompost of bovine manure

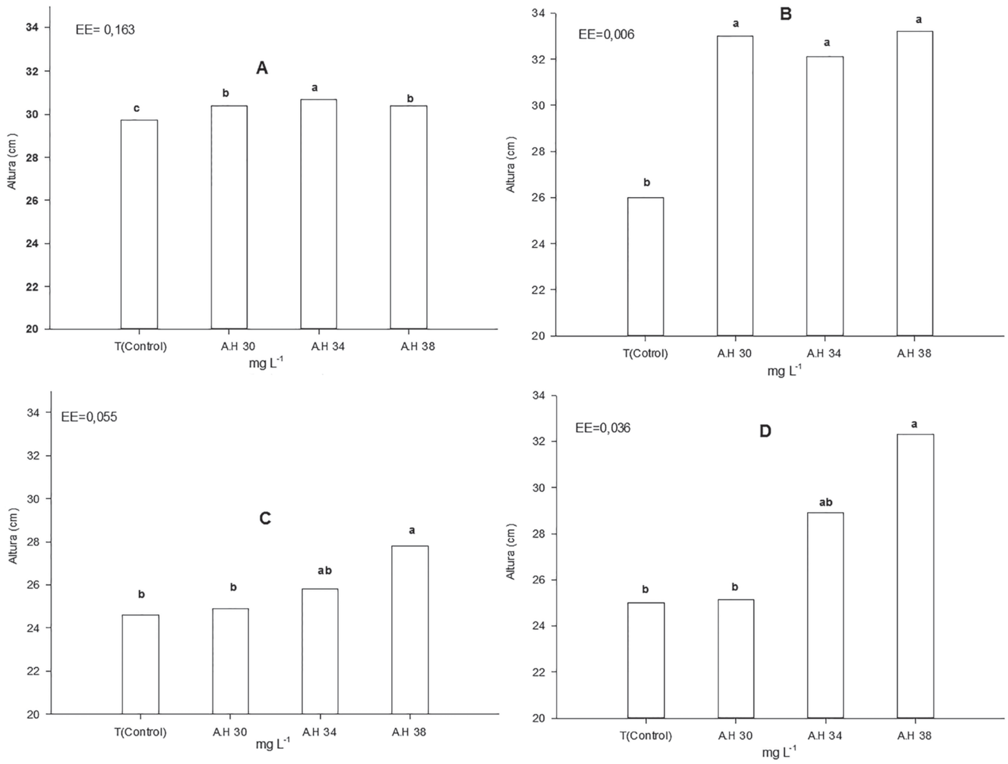

Figure 3. Height of rice plants of cultivars J-104 (A, C) and IACuba-33 (B, D) with foliar sprays of humic acids (AH) extracted from bovine manure, growing without (A, B) and with (C, D) effects of induced water stress

In Figure 3A the three concentrations used were able to stimulate the height in the cultivar J 104 for the condition of flooding, being the concentration of 34 mg L^{-1} the one that achieved the greatest significant stimulus with respect to the control.

It was also shown that in Figure 3B but now using the cultivar IACuba-33, it was found that also the three sprinkled concentrations reached a stimulus of up to 27 % higher than the control under said condition. It seems that the variety effect and its particular behavior are evident in these conditions, obtaining better results in the cultivar IACuba-33.

For both cultivars under flooding conditions (ideal environment for rice cultivation despite being a facultative hydrophytic plant), it was observed that there was a positive effect on the part of the AH on the growth in height when the AH are sprayed. (Figures 3A and B). This is physiologically possible if one takes into account the complex structure of the AHs as well as their richness in the functional groups, which could justify such behavior (19-21). Similar results were reported by other authors, working with the cultivar IACuba-30, under these same conditions (9).

As shown in Figure 3C for cultivar J 104 under conditions of water deficit, only one concentration managed to exert positive effects, reaching 12 % higher than the control. The trend shown was as follows, as the AH concentration increases, the height of the plant will increase. Similar results were found in Figure 3D with the cultivar IACuba-33, where the highest concentration reached a significant stimulus of 28 % higher than the control. These results differ from what was found when similar concentrations were applied on rice plants; however, the best effects were achieved by applying the lowest concentrations (7).

The seedlings of both cultivars in the water deficit condition reached a height at least similar to those of the control in flooding when the solutions of 38 mg L^{-1} were sprayed. Perhaps the effect of AH is determined by its action similar to other hormones that regulate defense mechanisms in the face of this type of stress.

A possible explanation for these effects could be supported on the basis of an action of AH equivalent to abscisic acid (ABA), which is chemically and physiologically possible taking into account that the action of this natural hormone is determined by its recognition by receptors hormonal, where the carboxyl groups of the aliphatic side chain, as well as the hydroxyl and carbonyls of ketonic nature play a fundamental role.

This type ABA effect could explain a stimulation in the synthesis of osmoprotective compounds in seedlings that act well as osmolytes (facilitating the retention of water by the cytoplasm and therefore readjusting the intracellular water potential (prolines, aquaporins), (21,22) and in addition, some stimulation may be occurring in the group of peroxidase enzymes (POX) which help to eliminate reactive oxygen species in excess, produced by the oxidative stress that is generated by the water deficit.

A good development of the root system for most crops and specifically the case of rice cultivation, favors a better and more efficient use of the mineral elements present in the complex soil, giving the plant greater potential for adaptability under adverse conditions, as they are the water deficit that in turn causes oxidative stress and the low availability of essential nutritional elements (23).

In Figure 4A cultivate J 104 it can be observed that under watering conditions and coinciding with the results obtained previously, two of the three sprinkled concentrations managed to overcome the control. In this case, the highest concentrations (34 and 38 mg L^{-1} AH) were those that achieved a significant stimulation, doubling the control. On the other hand, in Figure 4B for the cultivar IACuba-33 under this same condition and contrary to that observed in the cultivar J104, it was possible to appreciate that the stimulation was reached when applying the lowest concentration of 30 mg L^{-1}, reaching 46 % above the control.

Apparently under these conditions of flooding for the two cultivars used, the application of different concentrations of AH manage to stimulate the process of cell division and expansion which leads to the process of growth (irreversible increase in size or volume) and cell differentiation (process by which is achieved cellular specialization, so that the true development of the body is achieved) (24). Likewise these concentrations under these conditions, apparently also achieved that the photosynthetic process developed satisfactorily, reaching to produce the necessary metabolites to guarantee a significant increase in the production of biomass (6).

Results reported by other authors show that the most effective doses of humic substances, taken as reference to AH against water stress, vary between 20 and 46 mg L^{-1} AH for rice cultivation using other varieties (25,26).

Figures 4C and 4D cultivate J104 and grow IACuba-33 under conditions of water deficit, showed that in both cases the greatest stimulations of the dry root biomass became more evident when sprinkling the plants with the highest concentrations 34 and 38 mg L^{-1} AH, respectively.

The condition of water stress to which the crop was imposed did not significantly affect the development of both cultivars when the highest concentrations of AH were sprayed, and a protective effect of these compounds was observed under this condition. This behavior has been repeated in experiments performed by other authors on wheat (27) and on rice using different cultivars with concentrations equivalent to those tested (25).

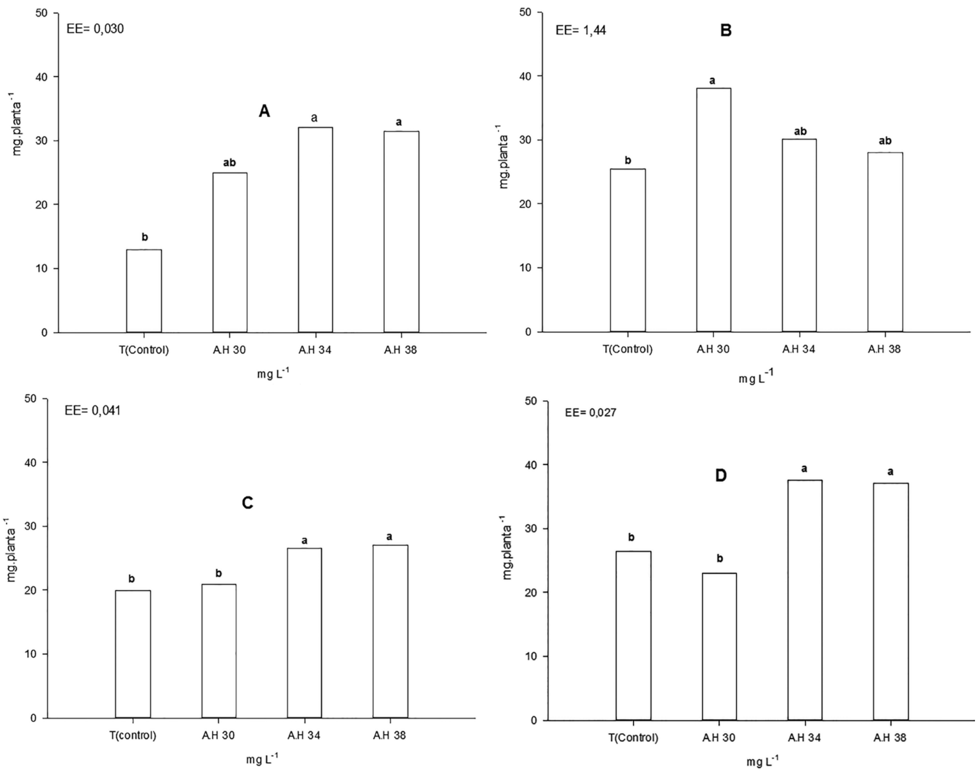

Figure 4. Root dry matter of rice plants of cultivars J-104 (A, C) and IACuba-33 (B, D) with foliar sprays of humic acids (AH) extracted from bovine manure, growing without (A, B) and with (C, D) induced water stress effects

In Figure 5A, culture J104 shows the average values obtained in the activity of peroxidases in organs of the aerial part of the plant under watering conditions. Under this condition, the lower concentrations did not significantly stimulate the specific activity of this enzyme; however it was found that with the treatment of higher concentration 38 mg L^{-1} if it significantly stimulated the activity of this enzyme system. In addition, in Figure 5B for the cultivar IACuba-33 it was observed that the concentrations 30 and 34 mg L^{-1} of AH produced an inhibitory effect on the enzymatic activity of the POX and the higher concentration, neither did it modify said enzymatic activity. Apparently under these conditions, the highest concentration would be the most suitable so that the activity of POXs does not decrease.

Similar results have been reported in studies of the cultivation of rice cultivar J-104 with and without stress (28), but by sprinkling foliar concentrations of up to 2 μmol L^{-1} of epibrassinolide. Other similar results have been reported working with rice plants exposing them to moderate periods of humidity and drought (29).

Figure 5C cultivar J104 shows the activity of the peroxidases, under water deficit conditions. The POX, under these conditions, act on reactive oxygen species in excess turning them into harmless species for plants, also participate in several essential metabolic processes including the regulation of cell growth, lignification, phenolic oxidation and defense against pathogens (30).

This figure shows that the lower concentrations of 30 and 34 mg L^{-1} of AH were not ideal to achieve this stimulation, with the highest concentration of 38 mg L^{-1} clearly showing values of enzymatic activity higher than obtained in the control plants.

In Figure 5D, cultivate IACuba-33 under conditions of water deficit, it could be corroborated that the lower concentrations are inefficient to achieve this stimulation. It was also observed that, as in Figure 5C, the best stimulation was achieved by sprinkling the highest concentration 38 mg L^{-1}.

As can be seen in Figure 6A, cultivate J104 under water conditions only the concentration of 34 mg L^{-1} was able to significantly increase the concentration of proteins, with 28 % higher than the control.

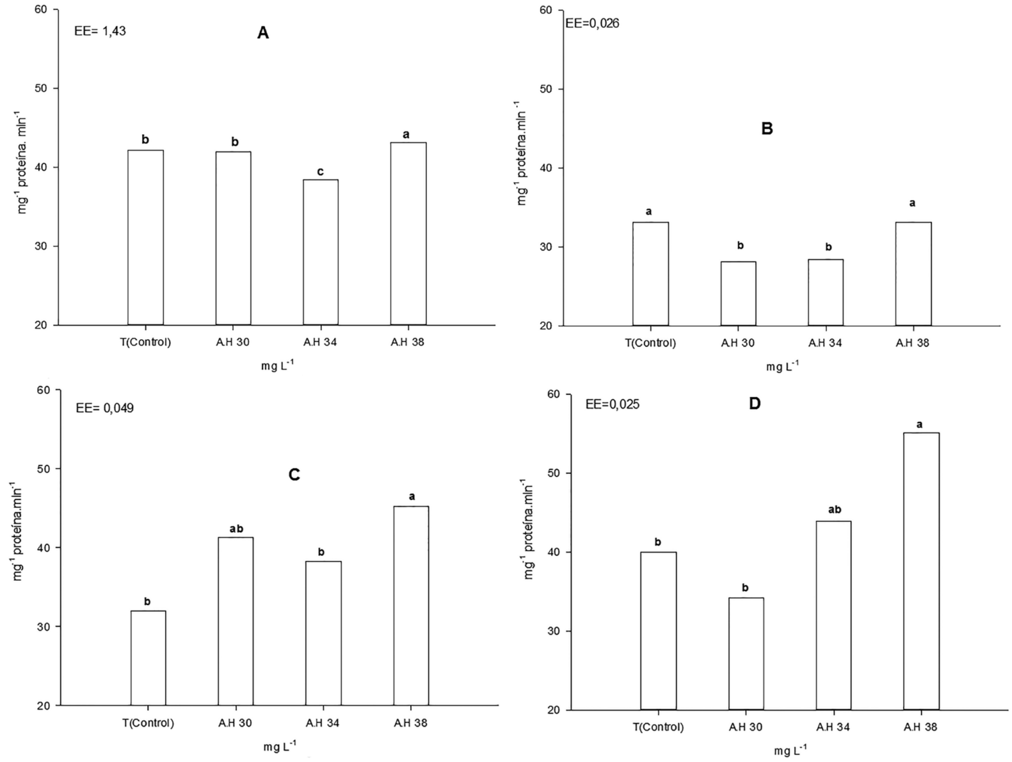

Figure 5. Peroxidase activity (POX) in rice plants of cultivars J-104 (A, C) and IACuba-33 (B, D) with foliar sprays of humic acids (AH) extracted from bovine manure, growing without (A, B) and with (C, D) induced water stress effects

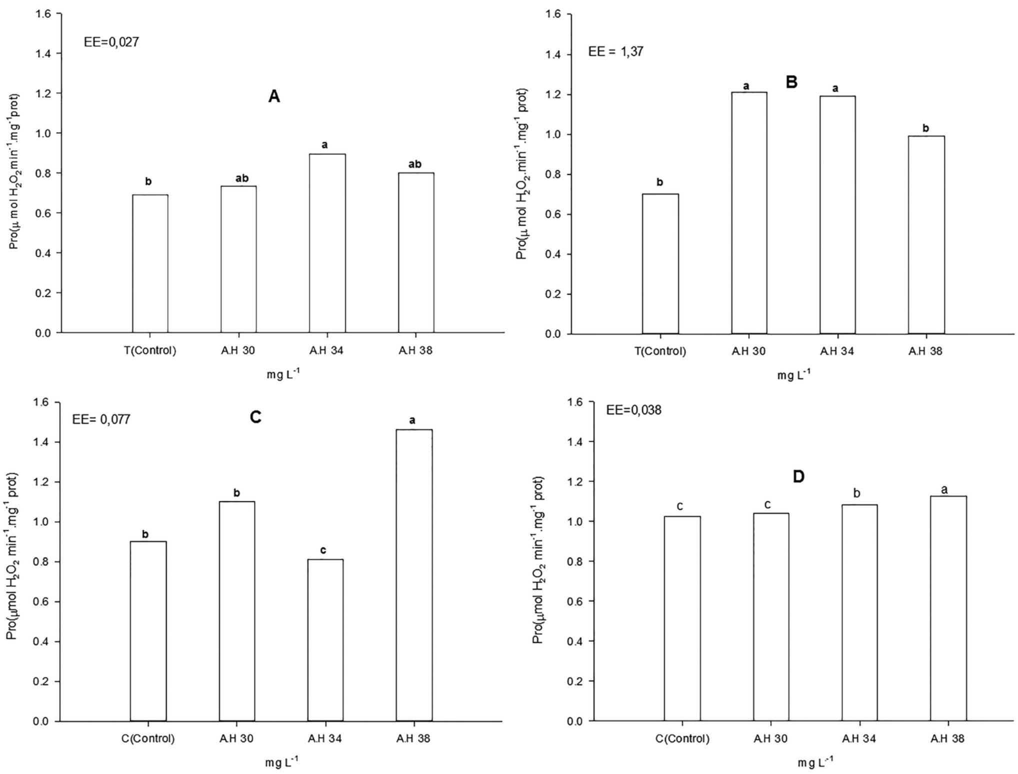

Figure 6. Total protein content in rice plants of cultivars J-104 (A, C) and IACuba-33 (B, D) with foliar sprays of humic acids (AH) extracted from bovine manure, growing without (A, B) and with (C, D) induced water stress effects

In Figure 6B cultivate IACuba-33 under this same condition it was observed that in this case the greatest increases in protein concentration were achieved by sprinkling the lowest concentration of 30 mg L^{-1} which achieved a marked stimulation of 41 % higher than control. The graph showed the following trend, as the AH concentration decreases, the concentration of proteins increases. Results similar to those obtained in this work, have been reported by other researchers conducting studies of the content of POX (25), aquoporins and protective osmolytes (proteins) under water conditions and water deficit in rice plants, where the total protein content was stimulated by the application of concentrations of humic substances up to 40 mg L^{-1}.

Likewise in Figure 6C it is observed that only the application of the highest concentration of 38 mg L^{-1} with 62 % superior to the control manages to cause a marked significant increase in the levels of total soluble proteins with respect to the control.

In Figure 6D using the cultivar IACuba-33 shows that the treatment four and three to which corresponds the highest concentrations 34 and 38 mg L^{-1} of AH managed to modify the concentrations of total soluble proteins with respect to the control, contrary to the obtained in this same cultivate in conditions of flooding.

The differences observed between one cultivar and another under water and water deficit conditions could be justified to a certain extent by the varietal characteristics and their particular behavior before these conditions. This explanation is supported by another author, who states that different rice cultivars have different efficiencies for water use and growth (31).

Likewise, the effect induced by the structural fragments of the AH on the different indicators evaluated under conditions of water stress and water deficit could be explained through the analogous action of these same structural fragments of the hormone-like AH that participate in the optimization of the plant defense process in these conditions. The abundance and diversity of these functional groups in AH allow and explain the observed physiological effects.

A possible hypothesis for the explanation of the protective effect of AH under water deficit condition could be supported on the basis of the action of AH type abscisic acid (ABA) which is possible if it takes into account the principle of action of this natural hormone.

This type ABA effect (23), could explain a stimulation in the synthesis of osmoprotective compounds in seedlings that act well as osmolytes (facilitating the retention of water by the cytoplasm and therefore readjusting the intracellular water potential (prolines, aquaporins) and also some stimulation may be occurring in the group of peroxidase enzymes (POX) which help to eliminate active oxygen species produced in excess by the oxidative stress that is generated by the water deficit.

CONCLUSIONS

- The extracted AH present similar characteristics to the standards of the International Humic Substances Society (IHSS). UV-Vis spectroscopy shows a high presence of aromatic structures and quinones, while the E_4/E_6 ratio justifies a high degree of aromatic condensation. The FTIR spectra show the presence of several characteristic oxygenated functional groups (OH, COOH, C = O), which are similar to those of natural compounds in plants that exert hormonal activity.
- The different concentrations of AH that were sprayed in both cultivars in the beginning of tillering stage, stimulate the different indicators evaluated.
- In J-104 cultivar, the concentrations of 34 and 38 mg L^{-1} of AH are the ones that have the greatest effect on the physiological indicators evaluated (height of the plant and dry mass in the radical part, in both conditions). These same concentrations are those with the best effects on the two biochemical indicators used (peroxidase activity and total content of soluble proteins in both conditions).
- For the IACuba-33 cultivar, the best results are also obtained when the plants are sprinkled with the concentrations of 34 and 38 mg L^{-1} of AH. The physiological indicators that show these results are height of the plant for both conditions and dry mass in the radical part in water deficit condition, while for the biochemical variables are the total content of soluble proteins and the specific activity of peroxidases, in both terms. Unlike the effect induced in the cultivar J-104, the concentration of 30 mg L^{-1}, sprinkled in the cultivar IACuba-33, stimulates the indicators of height of the plant, dry mass in the radical part and soluble protein content under condition of flooding.

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