Cultivos Tropicales, 2022, vol. 43, no. 2, e09
ISSN impreso: 0258-5936
ISSN digital: 1819-4087

http://ediciones.inca.edu.cu

abril-junio
Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas

Original article

Effect of a vermicompost extract on *Lactuca sativa* L. grown under low water supply

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ABSTRACT

Agriculture, a highly water-consuming sector, uses about 70 % of potable water for irrigation, which affects the availability of this scarce and limited resource. For decades, the biostimulant action of aqueous extracts of humic substances from cattle manure vermicompost has been demonstrated in numerous crops, and the protective effect against abiotic stress conditions has also been proven. Lettuce (*Lactuca sativa* L.) is a water-demanding vegetable that responds to the stimulating effect of humic extracts. For these reasons, the aim of this work was to evaluate the effect of the application of a humic extract of vermicompost (HEV) on lettuce plants grown under low water supply. Experiments were conducted under controlled conditions, treating the plants with a combination of three dilutions of the obtained HEV and two water conditions (25 and 50 % of the maximum water holding capacity (MC), following a completely randomized experimental design. At 42 days after plant emergence, biological productivity indicators and biochemical-physiological indicators were evaluated. The humic extract of vermicompost presented advantageous physicochemical properties for use as a biostimulant. Plants sprayed with dilutions of the extract and under conditions of low water supply showed similar or higher values than the control with adequate water supply (MC 85 %). Increases in proline and leaf protein content were observed, but not in relative chlorophyll content. It is recommended to perform the total characterization of vermicompost extracts and to study the response of lettuce under field conditions.

Key words: humic substances, water availability, vegetables, productivity

INTRODUCTION

According to the report of the Food and Agriculture Organization of the United Nations (FAO) [(1)], agriculture is a highly water-consuming sector, using about 70 % of the planet's drinking water for crop irrigation. The percentage of drinking water present on the Earth's surface is only 0.025 % of the total, hence the need to make better use of this scarce and limited resource. It is also known that the world population has increased considerably, which brings with it an increase in the demand for agricultural products and in the extraction of water for irrigation [(2)].

In this context, the updating of the Cuban economic model aims at the development of sustainable agriculture, using an integrated management of science, technology and environment, taking advantage of and strengthening the country's available capacities [(3)]. In the country's economic and social strategy for boosting the economy and confronting the world crisis generated by COVID-19, there is a need to encourage the efficient use of material resources and savings as premises for the economy growth [(4)].

In the current context of Cuban agriculture, it is necessary to use bioproducts of natural origin, easily available, non-polluting to the environment, obtained through methodologies that reduce production costs and increase crop productivity, reducing the use of fertilizers and chemical pesticides imported at high prices [(5)].

The use of biostimulants in agriculture, a category that includes humic substances [(6)], has gained special attention as it provides numerous benefits in growth stimulation and protection against stresses [(7)], which contributes to the adaptation of agricultural production systems to the new scenario [(8)]. Researchers from the Department of Chemistry of the Agrarian University of Havana (UNAH), for more than two decades, have obtained aqueous extracts of vermicompost derived from cow manure based on humic substances (humic and fulvic acids) and verified the biostimulant action through the foliar application of these humates, enhancing biological and agricultural productivity in numerous crops of agronomic interest [(8-13)]; among them, lettuce (*Lactuca sativa* L.), corn (*Zea mays* L.), tomato (*Solanum lycopersicum* L.), chard (*Beta vulgaris* L.) and garlic (*Allium sativum* L.). The first of these crops corresponds to the most consumed leaf vegetable in the world, due to its content of vitamins and easily absorbed mineral salts, which is extremely sensitive to water deficit, due to its shallow root system [(14)], leading the grower to a water supply in excess of this crop needs [(15)].

Numerous researchers have addressed the potential of humic acids (HA) to produce defense mechanisms in plants that are subjected to abiotic stress, providing superior adaptive responses [(16-21)]. These adaptive responses to stress conditions such as salinity, drought, heavy metals, and even HAs themselves were explored at the transcriptional level [(21)], postulating that HAs can be used as a "*primed state*" (PS) agent of plant defense.

Taking into account the need for a more efficient use of water resources and the potential of using humic substances in plants grown under stress conditions, the aim of this research was to evaluate the effect of a humic extract of vermicompost (HEV) on lettuce plants grown under low water supply.

MATERIALS AND METHODS

Obtaining and characterization of vermicompost humic extract (HEV) from cattle manure

The cow manure vermicompost (CV) used to obtain the extract is marketed by the UEB various productions belonging to the Ministry of Agriculture (MINAG). The sieved (2 mm) and dried CV was subjected to extraction with a basic solution composed of KOH, Urea and KH_2PO_4, in a 1:10 (m:v) ratio [22] with some modifications [23].

The characterization of the extract obtained was carried out in the laboratories of the Chemistry Department of the UNAH and the Agrochemistry Laboratory of the National Institute of Agricultural Sciences (INCA) and consisted of pH determinations (potentiometry), electrical conductivity (conductimetry), soluble organic carbon (SOC) [24], organic carbon as humic acids (OC_{HA}) [25], Na, K, Ca, Mg ions and P content [26].

Effect of HEV application on biological and biochemical-physiological productivity indicators in lettuce plants with low water supply

Experiments were carried out in the light room of the INCA physiology laboratory (T= 22.1-27.6 ºC and RH= 40-73 %). Certified lettuce seeds of the Black Seeded Simpson variety, previously disinfected with 1 % NaClO, were sown in a soil: vermicompost mixture in a 3:1 (m:m) ratio. The maximum water holding capacity (MC) of this substrate was determined [27]. The soil used for this substrate corresponds to an agrogenic Ferrallitic Red Leached soil (FRL) [28].

Ten seeds were sown per pot (63 in total), in which 85 % of the determined MC was maintained each day. At 9 days after emergence (DAE), a thinning was performed to leave the two most homogeneous positions in each pot, which remained in these conditions for another seven days. From this moment on, irrigation was reduced so that the pots reached water supply conditions of 50 and 25 % MC, leaving a group of pots with 85 % MC, which corresponds to the optimum condition for this crop. After 16 DAE, the pots were subdivided following a completely randomized experimental design, performing foliar spraying of dilutions of the extract (1:40, 1:60 and 1:80, v:v), maintaining a group of pots with 85 % MC without spraying, which would function as a control. Fifteen days after the spraying of the HEV dilutions, with a conical hand sprayer and a dose of 5 mL per plant, another spraying was carried out under the same conditions. The treatment scheme is shown in Table 1.

Table 1. Description of treatments applied to lettuce plants under controlled conditions

Treatment	Significance
T1	85 % of MC (Control)
T2	25 % of MC and HEV spraying (1:40)
T3	25 % of MC and HEV spraying (1:60)
T4	25 % of MC and HEV spraying (1:80)
T5	50 % of MC and HEV spraying (1:40)
T6	50 % of MC and HEV spraying (1:60)
T7	50 % of MC and HEV spraying (1:80)

At 42 DAE, destructive and non-destructive sampling was carried out to evaluate the biological productivity indicators previously designed, using the nine replicates existing for each treatment. The number of leaves was determined by visual counting, plant height (cm) by measurement from the stem base to the upper leaf apex and root length (cm) by measurement from the collar base to the apical part of the main root, both with a graduated ruler of 1 mm precision. The fresh mass (MF) of the aerial part (g) was evaluated directly by weighing on analytical balance model (Sartorius BS-124S) d=±0.0001 g, and the leaf and root dry masses (g) were evaluated by gravimetry with the use of an air circulation oven (Venticell-707) at 60 ºC.

The third leaves of each plant of the same treatment were homogeneously mixed to perform the determinations of all biochemical-physiological indicators, which were performed in triplicate. The third expanded leaf of each plant in the pot was sampled [(29)] 42 days after emergence.

Leaf protein content was determined spectrophotometrically at 500 nm [(30)] using bovine serum albumin (BSA) as standard and was expressed as µg of protein g^{-1} FM. Relative chlorophyll content was determined with a portable chlorophyll meter (model SPAD-502, Minolta) on the third expanded leaf of each plant, taking the average of four measurements per leaf. The determination of proline as an indicator of stress resistance was carried out with some modifications [(31)]. For this purpose, 0.2500 g of leaves were sampled. Extraction was carried out with distilled water close to boiling point and colorimetric determination was performed with the reagent acid ninhydrin at 520 nm. Proline concentration was expressed on a fresh mass basis (µmol proline g^{-1} FM).

Data were statistically processed using Statgrafics Plus version 5.1 software, performing a simple rank analysis of variance (ANOVA) and the Tukey 95 % multiple comparison of means test.

RESULTS AND DISCUSSION

Obtaining and characterization of vermicompost humic extract (HEV) from cattle manure

The humic extract of vermicompost (HEV) obtained has the dark coloration characteristic of extracts containing humic substances (HS). Table 2 shows the physicochemical properties of the extract evaluated in this work, which are within the range of values found in the literature [(32)].

The pH value of the extract is close to neutrality and the electrical conductivity (EC), as well as the total soluble solids (TSS) present values that ensure the non-aggressiveness of the extract with the environment (22).

Table 2. Physicochemical properties of the humic extract of vermicompost

Property	pH	EC (mS cm^{-1})	TSS (g L^{-1})	SOC (%)	OC_{HA}	P	Ca^{2+}	Mg^{2+} (mg L^{-1})	Na^{+}	K^{+}
	7,29	8,99	5,14	35,48	25,79	647,36	95,00	28,00	85,28	55,02
S.Ex±	0,01	0,06	0,04	0,93	1,27	25,00	2,89	1,00	1,20	1,74

Means of three replicates

EC: electrical conductivity, TSS: total soluble solids, SOC: soluble organic carbon content, OC_{HA}: carbon content as humic acid

The SOC content of the extract obtained is 35.5 %, higher than that reported by another author (23), using the same extraction procedure, but different cow manure vermicompost. The contents of SOC in the extract, as well as that of OC_{HA} (25.8 %) are above the minimum values required (30 and 15 %, respectively) for the use of this type of liquid organic substances (32). It is reported that these values vary depending on the maturation time, source material and type of worm used to obtain the vermicompost (33).

With respect to the content of P, Ca, Mg, Na, and K elements, these differed considerably from those observed by other authors, who carried out a total characterization of humic extracts of vermicompost (22,34). The phosphorus content was lower than that obtained by another author (34), possibly because he used a different extractive mixture: $Na_4P_2O_7$/NaOH. The Ca^{2+} and Mg^{2+} contents obtained were much higher than those obtained in other studies (34), while the Na^{+} and K^{+} contents were lower than those reported by other researchers (22,34).

Thus, it is verified that the physicochemical properties of the HEV obtained differ from the properties of other extracts obtained (22,34), which is due to the use of different extraction protocols, as well as different CV whose natural variability causes extracts to present different composition (33); nevertheless, it is evident that the HEV obtained is a liquid extract rich in humic substances, with a high content of organic carbon and humic acids, which could exert a favorable effect on lettuce grown in conditions of low water supply.

Leaf emission was not affected in lettuce plants sprayed with different dilutions of HEV and subjected to different water supplies (Table 3). All treatments, with the exception of treatment T6 (HEV dilution 1:60 with 50 % water supply), did not show significant differences with respect to the control treatment (T1, 85 % MC). It should be noted that treatment T6 showed significantly higher values than T1 and T4 (HEV dilution 1:80 with 25 % MC), showing no differences with the rest of treatments. This result, taking into account that leaves are the organ of consumption in this crop, is particularly significant in plants subjected to the lowest water supply (25 %).

Table 3. Biological productivity indicators evaluated in lettuce plants sprayed with dilutions of vermicompost extract subjected to different water supplies

Treatments	Number of leaves (units)	Height plant (cm)	Root length (cm)
1	13,0000 bc	18,4444 c	20,4333 ab
2	14,3333 ab	19,7778 a	19,7611 ab
3	14,0828 abc	17,4778 bc	19,0922 b
4	12,8333 c	17,7333 bc	19,6933 ab
5	13,6667 abc	19,1533 a	19,1956 b
6	14,7778 a	17,6444 c	21,4133 a
7	13,8333 abc	17,3978 bc	21,4622 a
SE x	0,3386**	0,5512**	0,4592**

*Means with different letters indicate significant differences according to Tukey, p<0.05

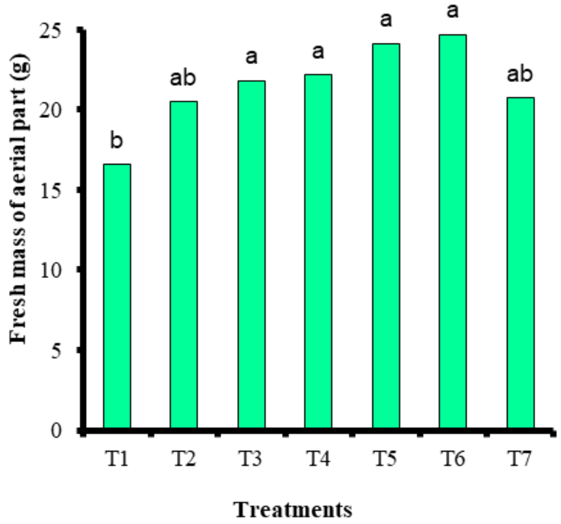
T1: 85 % MC (Control), T2: 25 % MC and HEV 1:40 spray, T3: 25 % MC and HEV 1:60 spray, T4: 25 % MC and HEV 1:80 spray, T5: 50 % MC and HEV 1:40 spray, T6: 50 % MC and HEV 1:60 spray, T7: 50 % MC and HEV 1:80 spray

Results for plant height show that it was possible to counteract the possible negative impact of insufficient water supply, through the foliar application of the dilutions of the CV extracts, achieving values in this indicator that did not differ from the control treatment, except for treatments T2 and T5 that presented significantly higher values, which correspond to the 1:40 dilution of the HEV with 25 and 50 % of the MC, respectively.

The positive effects of humic substance application on productivity indicators have been proven under water deficit conditions. It was found that rice seedlings under water deficit conditions and sprayed with a dilution of humic acid HA, reached a similar height to the control under waterlogged conditions (35), which coincides with the results of the present investigation. These results are promising considering that there is usually a significant reduction in plant height due to stress conditions.

When analyzing root length, a behavior similar to the other indicators was verified, since plants treated with foliar spraying of HEV dilutions and subjected to low water supply presented values similar to the control. The stimulation of root length under abiotic stress conditions has been documented. It was reported that this indicator in basil varieties (*Ocimum basilicum* L.) grown under moderate salinity conditions and with foliar application of 1:60 dilution of an extract of CV, presented values close to control plants (36). On the other hand, it was found that the application of HA of vermicompost on rice plants (*Oriza sativa* L.) caused a significant increase in root length under water stress conditions (37), showing an effect dependent on the concentration of humic acid used.

The application of dilutions of HEV was able to increase the fresh mass of the aerial part (Figure 1) in lettuce plants under conditions of insufficient water supply, presenting statistically similar or higher values than the control, although they did not show significant differences among themselves. The greatest increases were obtained with the 1:40 and 1:60 dilutions, with water supplies of 50 % (T5 and T6), followed by the 1:60 and 1:80 dilutions, with water supplies of 25 % (T3 and T4).



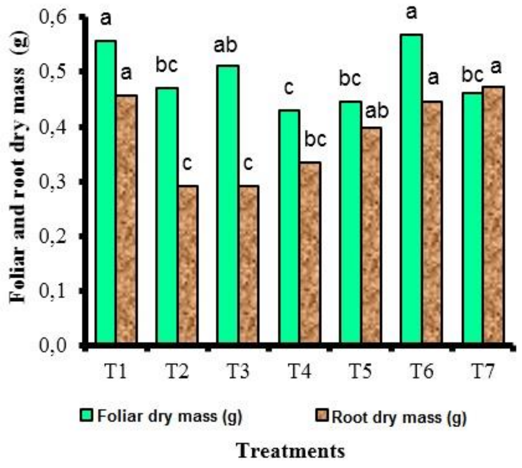
T1: 85 % MC (Control), T2: 25 % MC and HEV 1:40 spray, T3: 25 % MC and HEV 1:60 spray, T4: 25 % MC and HEV 1:80 spray, T5: 50 % MC and HEV 1:40 spray, T6: 50 % MC and HEV 1:60 spray, T7: 50 % MC and HEV 1:80 spray

*Means with different letters indicate significant differences according to Tukey, $p<0.05$

Figure 1. Fresh mass of the aerial part of lettuce sprayed with dilutions of vermicompost extract subjected to different water supplies

A stimulation was observed in the fresh mass of lettuce plants variety BSS-13 [9] that were sprayed with doses of Liplant®, a liquid humus with similar characteristics to the extract used in this work. Other authors report results for different crops and products based on humic substances that coincide with those observed in the present work [8-11,36,38]. On the contrary, no positive effects were found in this vegetable productivity, applying different doses of commercial humic acids to the soil [39], concluding that the application of HS is not effective in increasing the absorption of nutrients and crop productivity, which could be due to the form of application and dose used of the humic product.

When studying the foliar dry mass (Figure 2), it was found that there was a decrease in biomass, except for treatments T3 and T6, both with foliar spraying of the 1:60 dilution, which did not show significant differences with respect to the control. Thus, the spraying of the 1:60 dilution showed a favorable effect on the biomass production of plants cultivated with the lowest water supply, taking into account that biomass production in lettuce is significantly affected by the use of deficit irrigation [40].



T1: 85 % MC (Control), T2: 25 % MC and HEV 1:40 spray, T3: 25 % MC and HEV 1:60 spray, T4: 25 % MC and HEV 1:80 spray, T5: 50 % MC and HEV 1:40 spray, T6: 50 % MC and HEV 1:60 spray, T7: 50 % MC and HEV 1:80 spray

*Means with different letters indicate significant differences according to Tukey, p<0,05

Figure 2. Foliar and root dry mass of lettuce plants sprayed with dilutions of vermicompost extract subjected to different water supplies

Significant increases were found in the foliar biomass of American lettuce Raider Plus variety (41), with the application of different fulvic acid dilutions extracted from leonardite, proving that this effect was concentration dependent, similar to what was observed in the present work. The influence of the application dose, as well as the source of HS and, to a lesser extent, the type of plant and growth conditions, were investigated by analyzing different scientific articles (42), estimating increases of 22±4 % in the dry mass of sprouts and 21±6 % in the root dry mass in response to the application of these substances.

In relation to the radical dry mass, a decrease of this variable was observed in the treatments with respect to the control, except for those with supplies of 50 %, which coincides with a study carried out in rice cultivation (19), observing that the net production of radical biomass was affected in stress conditions, while when humic acids were applied, similar and superior results to those of the stressed plants were obtained.

The results obtained in these indicators demonstrate the beneficial effect of the foliar application of dilutions of the humic extract of vermicompost obtained, in lettuce plants with insufficient water supply, exerting a restorative effect on the Black Seeded Simpson variety similar to that found by other authors, working under abiotic stress conditions and in different crops (19,35,36). It is noteworthy that the T6 treatment, with the foliar application of the 1:60 dilution with 50 % MC, was the one that showed the best results in both foliar and root biomass production.

These effects could be explained by the hormonal activity of the HS contained in the extract and their ability to stimulate physiological, biochemical and nutritional processes under different conditions (43,44). In addition to hormonal mechanisms, others could be involved, such as nutritional mechanisms, with the improvement in the absorption and assimilation of nutrients (7,44) and defense mechanisms with the regulation of secondary metabolites and enzymes of the antioxidant system that regulate the content of reactive oxygen species (ROS) (19,45).

An increase in leaf protein content (Table 4) is evident with foliar spraying of HEV dilutions, mainly in those treatments where higher concentrations of the extract were used and with low water supply. Overall, there was a 26 % increase in protein content, which is evidence of a beneficial effect of the vermicompost extract dilutions on lettuce under low water supply conditions.

Table 4. Foliar biochemical-physiological indicators in lettuce plants sprayed with dilutions of vermicompost extract subjected to different water supplies

Treatments	Foliar Content of proteins (mg g^{-1} FM)	Relative chlorophyll content (SPAD)	Proline content (μmol g^{-1} of FM)
1	1,9941 c	7,5328	0,2713 c
2	2,6193 a	7,4933	0,4265 b
3	2,4059 a	7,4917	0,8379 a
4	2,3113 bc	7,4028	0,3995 b
5	2,1203 bc	6,7172	0,2866 c
6	2,4063 ab	7,0672	0,7609 a
7	2,4493 a	7,3083	0,4816 b
S.Ex	0,0630**	0,3047 NS	0,0219**

T1: 85 % MC (Control), T2: 25 % MC and HEV 1:40 spray, T3: 25 % MC and HEV 1:60 spray, T4: 25 % MC and HEV 1:80 spray, T5: 50 % MC and HEV 1:40 spray, T6: 50 % MC and HEV 1:60 spray, T7: 50 % MC and HEV 1:80 spray

*Means with different letters indicate significant differences according to Tukey, $p<0.05$

These results are equivalent to those found by other authors [35,36,43], where the HS application increased protein content in plants under abiotic stress conditions. This increase could be related to the activation and/or regulation of antioxidant systems, particularly enzymes that regulate the content of reactive oxygen species ROS, or it could be connected to the hormonal action exerted by HS, promoting growth and increasing the content of metabolites such as proteins and amino acids [43,46].

The increase in the activity of enzymes of the antioxidant system, as well as the content of proline and photosynthetic pigments has been observed with the foliar application of humic substances under conditions of water stress or drought [18,35]. Increases in protein content induced by humic substances present in a vermicompost extract, which was foliar sprayed on different crops, were observed [8,9]; this increase in lettuce was attributed to the activation of different enzymes related to the absorption and assimilation of nutrients and plant defense, also observed in beans under heavy metal stress conditions [18].

In relation to relative chlorophyll content (Table 4), it was observed that there were no differences in this indicator a few days after harvest. This suggests that the nutritional status of the plant, specifically the nitrogen and leaf chlorophyll content, was not modified by the application of deficit irrigation when using dilutions of the vermicompost extract, since several authors have found a correlation between the leaf chlorophyll content, the chlorophyll index in SPAD units and the total nitrogen content in various species [47].

With the AH application to the nutrient solution, photosynthetic activity increased in lettuce under normal humidity conditions [48], due to an increase in chlorophyll content in SPAD units. However, plants under stress conditions did not manage to overcome non-stressed plants in this indicator, and a similar behavior was observed in other indicators similar to that observed in the present work.

Leaf chlorophyll content showed an irregular behavior with the use of HS, showing increases or decreases according to the conditions used, according to a meta-analysis carried out on the use of humic substances, which could indicate that these results vary according to the source, extractor, concentration, plant species and crop conditions to which the ^{H}S are applied. These differences result in a variety of effects and responses on the part of plants with the application of humic substances (43), and a clearly stimulatory effect on this indicator cannot be established.

In relation to proline content (Table 4), it was observed that the response to foliar application of CV extract dilutions combined with water deficit was a significant increase in this indicator, with the exception of treatment T5. The treatments with the 1:60 dilution of HEV in both water conditions are highlighted, results that differ from those achieved in other investigations (36).

ABA application effects and water stress on lettuce were studied, observing an increase in proline content in both stressed and non-stressed plants (49). According to these authors, the increase in proline content of plants may be an adaptation to overcome stress, as it can serve as a compatible solute that helps tissues tolerate stress by contributing to increased osmotic potential and maintaining cell turgor, providing protection to cell membranes and walls, increased efficiency of photosynthesis, and scavenging of free radicals.

The increase in proline content observed in our work due to the application of the extract dilutions independently of the stress condition could be explained by the mimetic effect of ABA (35), which could be exerted by the humic substances present in this extract, which contributes to mitigate the adverse impact of stress and improve the growth, productivity and quality of plants (49-51).

As a whole, treatment with dilutions of a vermicompost extract rich in humic substances in lettuce of the Black Seeded Simpson variety with insufficient water regime had a beneficial effect on the indicators evaluated. It should be noted that the T6 treatment, with foliar application of the 1:60 dilution with 50 % MC was the one that showed the best results. When analyzing the results obtained in the biochemical-physiological indicators, it can be concluded that they were favorable, however, these results are not conclusive to give an integrative explanation, so it is necessary to examine other indicators.

The "hormone-like" effects of HS have been used to justify the beneficial effects on plant growth and development; however, it is recognized that multiple mechanisms are involved in the action that these substances exert, including signaling pathways that can be dependent or not on hormones such as indoleacetic acid (IAA) or abscisic acid (ABA) (46,48), the latter being the most related to the anti-stress response of HS (19). An increase in the concentration of hormones such as jasmonic acid in the roots and of cytokinins in the aerial part has been observed in plants treated by both foliar and radical routes, which is attributed to the induction of a superior response against pathogens in cucumber (*Cucumis sativus* L.) (52).

The results obtained are compatible with the fact that the beneficial action of humic acids applied via foliar or radical, can result in biochemical and molecular events activated by the moderate stress associated with the application of these substances [52]. Most of these works are carried out with isolated, purified and radically applied H[A], so that further research would be necessary to elucidate the biochemical-physiological events related to the beneficial action caused by the HEV foliar application on plant development, which contains HS as a major fraction.

CONCLUSIONS

- The humic extract of cow manure vermicompost (HEV) presents chemical and physicochemical properties that allow its use for agronomic purposes.
- It was proved, from the results obtained in the productivity indicators evaluated, that the foliar application of HEV dilutions promotes growth under conditions of low water supply.

RECOMMENDATIONS

- Characterize the extracts, specifically the coagulation threshold, the content of mineral elements, and the content of phenolic and carboxylic groups).
- To develop the study under organoponic or protected conditions of this crop, also testing other water conditions different from those used in this work.
- To determine other biochemical-physiological indicators in the lettuce crop under low water supply conditions.

ACKNOWLEDGMENTS

To the Master's program in Biofertilizers and Plant Nutrition of the National Institute of Agricultural Sciences (INCA). To the technicians and researchers of the Departments of Plant Physiology and Biofertilizers and Plant Nutrition (Agrochemistry Laboratory) of INCA, for their contribution in the execution of this work.

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