IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 12, Issue 2 Ver. II (February. 2018), PP 33-39
www.iosrjournals.org

Evaluation of Germination of Chilli Pepper Using Humic Substances And Humic Acids

Jadla Higino Vieira[1], Lennon Kledson dos Santos Silva[1],
Luciana Camargo de Oliveira [2], Janaína Braga do Carmo[2],
Luana Maria Tavares Rosa[2], Wander Gustavo Botero[1]*

[1] *Federal University of Alagoas, Graduate Program in agriculture and environment, Brazil.*
[2] *Federal University of São Carlos, Sorocaba Campus, Graduate Program in Biotechnology and Environmental Monitoring, Brazil.*
Corresponding Author: Jadla Higino Vieira

Abstract : *Humic materials used as organic fertilizer can promote seed germination and improve the quality of seedlings of vegetable crops. The objective of this study was to evaluate the effect of humic substances (HS) and humic acids (HA) extracted from peat samples on germination of Capsicum frutescens L. and its influence on seed quality and initial seedling development. The experiment was conducted in completely randomized design, with 6 treatments and 5 repetitions of 35 seeds for each treatment in a factorial 2 x 6, represented by 2 types of humic materials (humic substances and humic acids) and 6 doses with neutral pH (distilled water (control), 10 mg L^{-1}, 20 mg L^{-1}, 30 mg L^{-1}, 40 mg L^{-1} and 50 mg L^{-1}. The results showed that HS and HA have not inhibited the germination in C. Frutescens, indicating that the humic materials are effective to promote greater germination rate index (3.44), longer length of radicle (39.35 mm) and larger length of the aerial part of the seedling (28.66 mm) considering the dose 50 mg L^{-1} in 14 days after sowing.*

Keywords: *Capsicum Frutescens L., humic material, biostimulant.*

Date of Submission: 12-02-2018 Date of acceptance: 27-02-2018

I. Introduction

The main constituents of natural organic matter (NOM) are humic substances (HS) and their fractions, defined as humic acids (HA), fulvic acids (FA) and humin (HUM). The extraction and purification steps adopted by most researchers associated with the International Society of Humic Substances (IHSS) involve extended time and the use of acids and bases.

Fractions of NOM have been widely used in technological sectors, environmental and as a substrate for agriculture, seeking to increase productivity and decreasing chemical substrates [1, 2].

It is known that humic substances and their fractions exert hormonal activity in plants, but its mechanism is still unknown [3] and difficult to determine, mainly due to the chemical heterogeneity of humic substances.

Recent studies conducted by Traversa et al. [4] showed an increase in the germination of four switchgrass populations, after application of three differents concentrations of humic acids isolated from three different composts (green composts, mixed composts and coffee composts). Bernardes et al. [5], and the authors Canellas and Olivares [6] verified an increase in speed of germination rate of vegetables after application of humic acids.

Humic materials provide an increase in plant size and number of roots, nutrient uptake and growth rates, as well as greater rooting, influencing biochemical and physiological processes, increasing the assimilation of water by the seeds. These materials contributing to the efficiency in the enzymatic synthesis due to the formation of soluble compounds with micronutrients [7].

Studies with humic acids and humic substances applied for seed germination include evaluations of lettuce [8] soybean [9], sorghum [10], tomato [5] and wheat [7]. These authors are not unanimous in the use of these substrates because of the beneficial effects depend on a number of factors as characteristics of the Humic material, substrate concentration and type of plant species evaluated [5-10].

The chilli pepper (*Capsicum frutescens L.*), is a shrubby species with semi-ligneous stem and red fruits, characterized by the specific flavor and aroma determined by chemical components such as capsaicin and capsaicinoids [11]. This species of pepper is prominent in the world market of fresh vegetables, besides its use in the production of condiments, seasonings and preserves in several countries like India, Brazil and Thailand.

[12]. It is estimated that a quarter of the world's population consumes pepper fruits and its derivatives, presenting a strong performance in food, pharmaceutical and cosmetic industries [13].

Among the several studies carried out for the advance in the agricultural production of C. frutescens can be mentioned the studies of Mengarda and Lopes [14] on the initial development of chilli pepper plants; Torres and Borges [15] on the growth of pepper through the action of hormones and Rebouças et al. [12] about the physical-chemical characterization of this species in natura and processed. However, there are few studies using humic substances and humic acids as a substrate for initial seedling growth of chilli pepper [14, 15].

An interesting trend is the increasing interest of the agroindustry in the sale of extracts of OM from alkaline soil, especially those obtained from peat soils, as natural fertilizers to replace the commercially used inorganic fertilizers. The commercial version of "natural fertilizers" is generally available in the form of "wet" substances, its purification being similar to the IHSS procedure, often using other extractors, but without HCl / HF treatment and dialysis.

The objective of the present work was to evaluate the effect of the application of humic substances and humic acids extracted from peat samples on the initial germination and growth of *Capsicum frutescens L*. The impact on the physiological quality of seed and the initial seedling growth was also evaluated.

II. Materials And Methods

2.1. Sample collection and preparation of peat samples

The peat samples were collected in the municipality of Ribeirão Preto - SP (district of Taquaral-SP), at a soil depth of 50 cm, where five simple samples were collected to form a composite sample. These samples were transferred to wood trays and after drying in air for approximately 2 days, were passed in plastic sieves of 2 mm mesh.

2.2. Extraction of Humic substances and humic acids from peat samples

The extractions of humic substances (HS) and subsequent fractionation to obtain the humic acids (HA) were done according to the procedure adopted by most researchers associated with the International Society of Humic Substances (IHSS) with 0.1 mol L^{-1} (NaOH) as extractor in the ratio of 1:10 (peat:extractor) under inert atmosphere and agitation for 4 hours. The humic acids were obtained by fractionation of the humic substances with decreasing pH according to IHSS. The HS and HA solutions had the pH adjusted to 7.0.

2.3. Characterization of humic substances and humic acids

2.3.1. Elemental analysis

Samples of HS and HA were characterized by elemental analysis (C, H, N, O, S) in ThermoFiningan Flash EA1112 equipment. Standards used were: cystine, BBOT (2, 5-bis(5-tercbutyl-2-benzoxazol-2-yl)tiophen; methionine; sulfanilamide. All samples were analyzed in triplicate. The atomic ratios H / C and C / O were calculated from the data obtained in the elemental analysis.

2.3.2. Nuclear magnetic resonance of carbon 13 (NMR ^{13}C)

The functional groups content in the samples of humic substances and humic acids were determined by Nuclear magnetic resonance of carbon 13. The NMR^{13}C experiments with cross-polarization (CP) and magic-angle spinning (MAS) with variable amplitude (VA) were carried out in a Varian spectrometer (model Unity Inova 400). The samples were arranged in a cylindrical 5-mm diameter zirconium rotor (Doty Supersonic) spinning at 6 kHz in a Doty Supersonic probe for solid tests. TheNMR13CVACP/ MAS spectra were obtained under the following experimental conditions: resonance frequency of 100.05 MHz for 13C, spectral band of 20 kHz for cross-polarization, proton preparation pulse of 3.8 μs, contact time of 1 ms, acquisition time of 12.8 ms, and wait time of 500 ms for relaxation. The chemical shift values were referenced to hexamethyl benzene (HMB), which has a well-defined line at 17.2 ppm.

2.3.4. UV–vis spectroscopy: determination of the E4/E6 ratio

The E4/E6 ratio was ascertained by measuring approximately 2.0 mg of HA and HS samples in 10 mL of a $NaHCO_3$ 0.05 mol L^{-1} solution and later reading the absorbance at 465 and 665nm on a DR 3900 spectrometer [16].

2.4. Germination and initial growth experiments

The experimental design was a completely randomized design (CRD) with 5 replicates of 35 seeds for each treatment and 2 x 6 factorial scheme, represented by 2 humic materials (HS and HA) and 6 doses (distilled water (control), 10 mg L^{-1}, 20 mg L^{-1}, 30 mg L^{-1}, 40 mg L^{-1} and 50 mg L^{-1}) [17].

The seeds were uniformly distributed in germination boxes of 11 cm x 11 cm x 3.5 cm on two sheets of germitest paper moistened with 2.5 times the dry substrate weight and placed to germinate at 25 ° C. The germination percentage was evaluated in the first and last counts at 7 and 14 days after planting, respectively, considering germinated the seeds that provided seedlings with potential to continue their Normal growth, presenting root and aerial systems and coleoptile according to Brasil [17].

3.0 mL of the different solutions of the treatments were applied daily to the substrate using spray cans and the treatments without reapplication were re-moistened with the same amount of distilled water.

The Gerboxes were kept in Biochemical Oxygen Demand (B.O.D) germination chamber for a period of 14 days, in which they were evaluated: The germination percentage (G%); Germination rate (GR), mean germination time (MGT), Aerial part (AP), root length (RL). Subsequently, the data were submitted to analysis of variance and the means were compared by Tukey's test, at a 5% probability level ($p < 0.05$) and adjusted by linear or polynomial regression ($p \leq 5\%$) to explain the effects of the different levels of humic substances and humic acids on the germination of chilli pepper. The statistical analyses were performed using the software SISVAR, version 5.3 Build 77 [18].

III. Results

The results listed in Table 1 show that humic acids presented a higher degree of humification and aromaticity when compared to humic substances.

Table 1. Characterization results for samples of humic substances (HS) and humic acids (HA) of peat samples by atomic ratio H/C and C/O, functional group content determined by ^{13}C nuclear Magnetic Resonance (^{13}C NMR) and E4 / E6 ratio.

Parameter	Samples	
	HS	HA
H/C	1.16	1.20
C/O	0.81	0.96
Aliphatic (%)	23.7	34.0
Ether (%)	4.2	12.0
Aromatic (%)	61.6	23.0
Carboxylics, ethers And amide (%)	10.5	31.0
E4/E6	3.47	3.21

The use of different doses of humic acids and humic substances in the germination of C. frutescens showed that only the percentage of germination and Aerial part were not affected by the effect of the different humic materials (Table 2). On the other hand, there was a significant effect on germination velocity index, mean germination time and root length (Table 2).

In relation to root length and aerial part, it was observed that these variables were influenced by doses of humic materials (Table 2). Considering the statistical interaction between humic materials vs. Doses, significant results were observed only for mean germination time ($P<0.05$, $r=0.998$) and root length ($p<0.05$, $r=0.999$), evidencing the effect of humic materials depends on the doses (Table 2).

Table 2. Summary of variance analysis and mean germination percentage (% G), germination speed index (GSI), mean germination time (MGT), seedling radicular length (SRL), Aerial part (AP) of Capsicum frutescens at 14 days after sowing in the germination stage under different humic materials and doses.

Variation sources	GL	Valores de Quadrados Médios Average Square Values					
		% G	GSI	MGT	SRL	AP	
Humic Materials (M)	1	52.15^{NS}	0.82*	1.59*	616.69**	616.69**	
Doses (D)	5	42.90^{NS}	0.11^{NS}	0.26^{NS}	64.39*	13.13**	
Interaction (M vs D)	5	9.56^{NS}	0.25^{NS}	1.39**	64.18*	3.87^{NS}	
Linear Reg	1	16.29^{NS}	0.17^{NS}	0.88^{NS}	140.13*	22.64*	
Quadratic Reg	1	0.01^{NS}	0.13^{NS}	0.97^{NS}	16.12^{NS}	38.85**	
Cubic Reg	1	49.41^{NS}	0.01^{NS}	1.99**	163.41*	0.39^{NS}	
Deviation Regression	2	18.79 ^{NS}	0.11 ^{NS}	0.34 ^{NS}	1.14 ^{NS}	1.87 ^{NS}	
Residue	24	19.32	0.18	0.25	10.43	3.34	
VC(%)		4.78	12.84	5.24	11.76	7.02	
		Mean					
		(%)	(.......)	(days)	(mm)	(mm)	
Dose1 (Control)		90.95	3.97	8.55	27.51	26.54	
Dose 2 (10 mg L-1)		86.03	3.30	9.45	22.44	24.49	
Dose 3 (20 mg L-1)		90.00	3.19	10.19	25.05	24.97	
Dose 4 (30 mg L-1)		96.03	3.50	9.31	28.72	25.53	
Dose 5 (40 mg L-1)		92.06	3.46	9.25	31.65	26.18	
Dose 6 (50 mg L-1)		90.00	3.19	9.75	29.33	28.66	

* and **Significant at 5 and 1% probability, respectively, NS not significant.

In the evaluation of the germination speed, the application of humic acids presented an increase of 9% against the application with humic substances (Fig. 1).

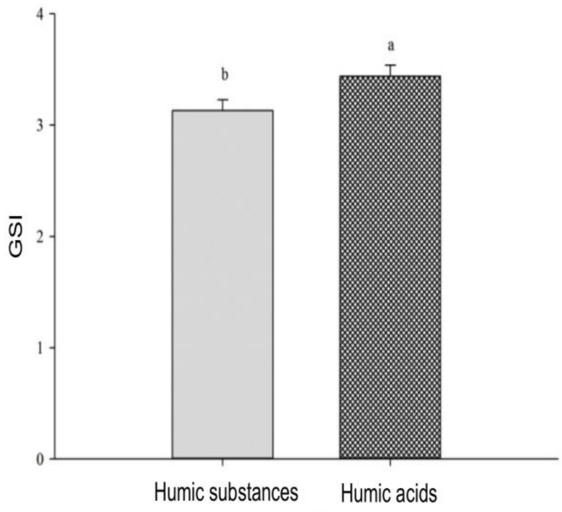

Figure 1. Germination speed index (GSI) at 14 days after sowing in function of humic substances and humic acids in the germination phase of C. frutescens.

When evaluating the deployment of humic materials interaction within each dose, it was verified that from dose 4 (30 mg L^{-1}) there was a significant effect, with a progressive increase of humic acid values up to 50 mg L^{-1}. The radicle length was increased by 25% when using humic acids (Table 3). These results corroborate the study of Traversa et al. [2], when verifying that the root length was significantly altered by the addition of humic acids.

Table 3. Interaction (M vs D) of the humic substances (HS) and humic acids (HA) materials within each category of doses considering seedling radicle length (SRL) of C. frutescens. At 14 days after sowing in the germination stage.

Doses	Humic materials	
	HS	HA
1	18.02 a	21.99 a
2	20.02 a	24.87 a
3	22.11 a	27.97 a
4	24.64 b	32.80 a
5	25.73 b	37.58 a
6	29.31 b	39.35 a

means followed by the same letter do not differ in lines by tukey test at 5% probability.

About the aerial part, a quadratic adjustment was observed with increasing doses of humic materials (Fig. 2) and positive increments were observed from dose of 20 mg L^{-1}. With this, higher values (28 millimeters) were obtained with the application of 50 mg L^{-1}.

.

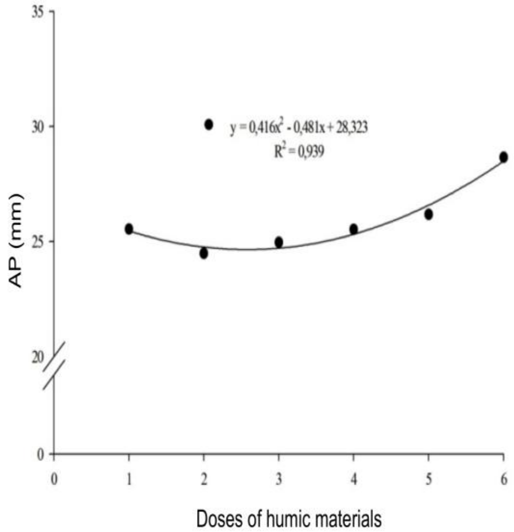

Figure 2. Aerial part (AP) of the seedling at 14 days after sowing as a function of the doses of humic materials in the germination stage of *C. frutescens*

IV. Discussion

One of the main factors that may affect seed germination and initial growth after application of humic substrates refers to the characteristics of organic matter fractions.

The atomic ratios determined by elemental analysis provide evidence of the structural characteristics of humic substances and humic acids. A lower H/C ratio indicates a higher aromaticity and a lower C/O ratio indicates a lower degree of humification of the NOM fractions. Giovanela et al. [19] suggests that H/C atomic ratios greater than 1 indicate that the origin of organic matter is due to vascular plants.

The contents of functional groups determined by ^{13}C NMR, corroborate with the H/C and C/O ratios, evidencing higher abundance of aliphatic and carboxylic groups in humic acids compared to humic substances. The E4/E6 ratio is an important indicator of condensation degree of humic molecules and is generally associated with aromaticity. According to the literature, ratios less than 4 are indicative of the presence of condensed aromatic structures and ratios greater than 4, indicate the absence of such structures [2, 20].

The use of different doses of humic acids and humic substances in the germination of C. frutescens are in agreement with Conceição et al. [21], who did not observe significant difference in the germination and vigor of corn seeds with the presence of humic material.

Piccolo et al. [22] evaluated the effects of humic acids and fractions of humic acids derived from coal on the germination and growth of the Lactugasativa and Lycopersicum esculentum species, and the data corroborate with this study, where there was no effects of humic materials application in the percentage of germination and also verified that the humic acids without fractionation presented better effects on the seedlings growth.

Borsari [23] emphasizes that these substances represent a source of Nitrogen, Phosphorus and Sulfur release, directly influencing plant nutrition and consequently the development of species. On the other hand, the humic acids contribute in the availability of the adsorbed or complexed phosphorus with ions in the clay fraction, forming the calcium phosphate precipitate that is unavailable to most plants.

Silva et al. [24] and Borsari [23] relate the promotion of root growth of species after application of humic materials with the concentration and origin of the material used in the soil and with the botanical species to be studied.

Piccolo et al. [22] shows that fractions of organic matter with low molecular weight and high levels of acid groups favor seedling development and cell elongation. The characterization data of the humic materials showed that humic acids have higher acid groups than humic substances. In addition, humic acids have lower molar mass compared to humic substances, which may be related to the increase in germination speed [22].

Bernardes et al. [5] demonstrated that humic substances can influence the increase and speed of germination rates and the growth of seedlings with better quality. According to Carvalho and Nakagawa [25] factors such as structure, aeration, water retention capacity and degree of contamination by pathogens may vary according to the humic material used, favoring or impairing the germination of the seeds.

Similar results, for the quality of the germination, were obtained with the humic substances isolated from soil organic matter where the humic substances used favor the development of the root system as well as the accumulation of nutrients and chlorophylls biosynthesis [26].

The mean time and the rate of germination are the main variables when it is aimed to evaluate the physiological potential of seeds. In this case, the main physiological effect of humic materials is the increase in root growth, which depends on the species and age, as well as on the source and concentration of humic material used [24].

According to Silva et al. [24] the analysis of the mean germination time is important to evaluate how the experimental conditions can influence in the speed of seed imbibition.

In the case of chilli pepper, the combination of materials that function as organic fertilizers, such as humic substances and humic acids, may result in different responses in the germination phase and in the initial development stage of the C. frutescens seedling [27].

Studying the direct interaction between acids and root growth, Jindo et al. [28] observed that in the root samples, which humic acids were applied, there was root growth, especially in samples containing more carboxylic groups. When analyzing the structural characteristics (Table 1) of humic acids against humic substances, we observed a higher content of carboxylic groups in humic acids, corroborating the results obtained for root growth.

Silva et al. [24] evaluated the application of different humic materials in *Solanum lycopersicum L.* (tomato) isolated from peat bogs at different stages of organic matter decomposition. The humic acids stimulated the appearance of root hairs, promoting a significant increase in the number of lateral roots emerged from the main axis. In this case, the humic acids were the humified fraction of greater bioactivity, since it presented greater capacity of induction of lateral roots in the initial stage of development.

This action may be associated with the performance of humic materials in the seedlings as a plant regulator, promoting an increase in H^+ synthesis and favoring, through the formation of an electrochemical gradient, the loosening of the cell wall and, consequently, expansion of cells, tissues and organs.

Thus, the presence of functional clusters with hormonal characteristics and the ability to activate the proton pump of the plasma membrane, are potential indicators of the bioactivity of humic acids [28]. These results corroborates with the structural characteristics determined in this study (Table 1).

In this context, Traversa et al. [2] comparing the main chemical and physico-chemical properties of humic acids at doses 10 mg L^{-1}, 50 mg L^{-1} and 200 mg L^{-1} and evaluating bioactive effects on the germination of *Panicum virgatum L.* observed that humic acids positively influenced germination and root growth.

While Vendruscolo et al. [10] evaluating the germination and initial growth characteristics of sorghum sorghum (*Sorghum bicolor L. Moench*) by applying a commercial product based on humic substances, verified that there was no effect of doses of humic substances on germination and initial growth of sorghum seedlings. In addition to these bioactive effects, humic acids are considered as additives for the introduction of microorganisms in the form of inoculants in the soil-plant system [6].

Thus, the effect of humic materials depends on the origin and quality of organic matter, important in the final relationship between organic acids and mineral, chemical and biological components from soil, promoting better and more stable interactions.

These results evidenced the possibility of using humic substances and humic acids in the germination of *Capsicum frutescens L.* as a natural fertilizer avoiding the use of substrates that may cause environmental impacts when applied.

V. Conclusion

Humic substances and humic acids from peat are promising in the germination and initial growth of Capsicum frutescens L. Although a further step in the extraction process was required, humic acids had more humification degree with higher contents of oxygenated groups when compared with humic substances. The humic acids reflected positively on the germination speed index (GSI) and mean germination time (MGT) of Chilli pepper seeds at 14 days after seeding. The application of humic acids also showed higher lengths for the radicle and aerial part of plant, considering the initial stage of development. Thus, the use of humic acids is more efficient for initial germination and growth of chilli pepper (Capsicum frutescens L.) compared to humic substances.

References

[1]. E. Loffredo, A.J. Palazzo, N. Senesi, C.E. Clapp, T.L. Bashore, Germination and Early Growth of Slickspot Peppergrass (Lepidium papilliferum) as Affected by Desert Soil Humic Acids, *Soil Science*, 175, 2010, 186-193.

[2]. A. Traversa, E. Loffredo, C.E. Gattullo, A.J. Palazzo, T.L. Bashore, N. Senesi, Comparative evaluation of compost humic acids and their effects on the germination of switchgrass (Panicum vigatum L.), *Journal of Soil Sedimentent, 14*, 2014, 432-440.

[3]. B. Šerá, F. Novák, The effect of humic substances on germination and early growth of Lamb's Quarters (Chenopodium album agg.), *Biologia, 66*, 2011, 470-476.

[4]. A. Traversa, E. Loffredo, C.E. Gattullo, A.J. Palazzo, T.L. Bashore, N. Senesi, Comparative evaluation of compost humic acids and their effects on the germination of switchgrass (Panicum vigatum L.), *Journal of Soil Sedimentent, 14*, 2014, 432-440.

[5]. J.M. Bernardes, J.M.R. Reis, J.F. Rodrigues, Effect of humic substance application on tomato seedlings, *Global Science Technology, 4,* 2011, 92-99.

[6]. L.P. Canellas, F.L. Olivares, Physiological responses to humic substances as plant growt promoter, *Chemical and Biological Technologies in Agriculture, 1,* 2014, 1-11.

[7]. L.F.O.S. Rodrigues, V.F. Guimarães, M.B. Silva, A.S. Pinto Junior, J. Klein, A.C. Costa, Características agronômicas do trigo em função de Azospirillum brasilense, ácidos húmicos e nitrogênio em casa de vegetação, *Revista Brasileira de Engenheria Agrícola e Ambiental, 18,* 2014, 31-37.

[8]. P.S.G. Bezerra, L.C. Grangeiro, M.Z. Negreiros, J.F. Medeiros, Use of biostimulant in the production of lettuce seedlings, *Científica, 35,* 2007, 46-50.

[9]. V.M. Benites, J.C. Polidoro, C.C. Menezes, M. Betta, Foliar application of organo mineral fertilizer and solutions of humic acid in soybean under no-tillage, *Embrapa, 35,* 2006, 1-6.

[10]. E.P. Vendruscolo, O.F. Santos, C.Z. Alves, Substâncias húmicas na qualidade fisiológica de sementes de Sorgo, *Journal of Agricultural Science, 3,* 2014, 169-177.

[11]. C.M.F. Pinto, C.L.O. Pinto S.M.L. Donzeles, Pimenta Capsicum: propriedades químicas, nutricionais, farmacológicas e medicinais e seu potencial para o agronegócio, *Revista Brasileira Agropecuaria Sustentavel, 3,* 2013, 108-120.

[12]. T.N.H. Rebouças, R.M.V. Valverde, H.L. Teixeira, Bromatologia da pimenta malagueta in natura e processada em conserva, *Horticultura Brasileira, 31*, 2013, 163-165.

[13]. H.W. Silva, L.M. Costa, O. Resende, D.E.C. Oliveira, R.S. Soares, L.S.R. Vale, Higroscopicidade das sementes de pimenta (Capsicum chinense L.), *Revista Brasileira de Engenharia Agrícola e Ambiental, 19,* 2015, 780-784.

[14]. L.H.G. Mengarda, J.C. Lopes, Qualidade de sementes e desenvolvimento inicial de plântulas de pimenta malagueta e sua relação com a posição de coleta de frutos, *Revista Brasileira de Sementes, 34,* 2012, 644-650.

[15]. R.C. Torres, K.C.A.S. Borges, Ação da giberelina no crescimento de pimenta (Capsicum frutescens), *Cadernos do Centro Universitário de Volta Redonda – UniFOA, 8,* 2013, 11-16.

[16]. R. Andreas, J. Zhang, Characteristics of adsorption interactions of cadmium(II) onto humin from peat Soil in freshwater and seawater media, *Bulletin of Environmental Contamination Toxicology, 92*, 2014, 352-357.

[17]. Brasil, Ministry of Agriculture, Livestock and Supply, *Rules for seed analysis, Mapa/ACS,* Brasília, 2009, 399 p

[18]. D.F. Ferreira, Sisvar: a computer statical analysis system, *Ciência e Agrotecnologia, 35,* 2011, 1039-1042.

[19]. M. Giovanela, J.S. Crespo, M. Antunes, D.S. Adamatti, A.N. Fernandes, A. Barison, C.W.P. da Silva, R. Guegan, M. Motelica-Heino, M.M.D. Sierra, Chemical and spectroscopic characterization of humic acids extracted from the bottom sediments of a Brazilian subtropical microbasin, *Journal of Molecular Structure, 981,* 2010, 111-119.

[20]. A.H. Rosa, I.C. Bellin, D. Goveia, L.C. Oliveira, R.W. Lourenço, N.L.D. Filho, P. Burba, Development of a new analytical approach based on cellulose membrane and chelator for differentiation of labile and inert metal species in aquatic systems, *Analytica Chimica Acta, 567*, 2006, 152-159.

[21]. P.M. Conceição, H.D. Vieira, R.F. Silva, S.C. Campos, Germinação e vigor de sementes de milho recobertas e viabilidade do inoculo durante o armazenamento, *Ciência e Agrotecnologia, 33,* 2009, 765-772.

[22]. A. Piccolo, G. Celano, G. Pietramellara, Effects of fractions of coal-derived humic substances on seed germination and growth of seedlings (Lactugasativa and Lycopersicumesculentum), *Biology and Fertility of Soils, 16,* 1993, 11-15.

[23]. F. Borsari, Humic substances. Field experiments demonstrate the benefits to the productivity of the use of humic acids in intensive agriculture, *Agro DBO, 15,* 2013, 44-44.

[24]. A.C. Silva, L.P. Canellas, F.L. Olivares, L.B. Dobbss, N.O. Aguiar, D.A.R. Frade, C.E. Rezende, L.E.P. Peres, Promoção do crescimento radicular de plântulas de tomateiro por substâncias húmicas isoladas de turfeiras, *Plant Soil, 353*, 2011, 209-220.

[25]. N.M. Carvalho, J. Nakagawa, *Sementes: ciência, tecnologia e produção* (Jaboticabal, SP: FUNEP, 2000).

[26]. L.P. Canellas, J.G. Busato, L.B. Dobbss, M.A. Baldotto, V.M. Rumjanek, F.L. Oivares, Soil organic matter and nutrient pools under long-termnon-burning management of sugar cane, *European Journal of Soil Science, 61,* 2010, 375-383.

[27]. M.A. Dias, J.C. Lopes, N.B. Corrêa, D.C.F.S. Dias, Germinação de sementes e desenvolvimento de plantas de pimenta malagueta em função do substrato e da lâmina de água, *Revista Brasileira de Sementes, 30,* 2008, 115-121.

[28]. K. Jindo, S.A. Martim, E.C. Navarro, F. Pérez-Alfocea, T. Hernandez, C. Garcia, N.O. Aguiar, L.P. Canellas, Root growth promotion by humic acids from composted and non-composted urban organic wastes, *Plant Soil, 353,* 2012, 209-220.

Jadla Higino Vieira. " Evaluation of Germination of Chilli Pepper Using Humic Substances And Humic Acids." IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 12.2 (2018): 33-39.