PROMOTER AND PHYTOTOXIC EFFECTS OF ETHANOLIC EXTRACTS OF CHILTEPIN IN THE ARABIDOPSIS THALIANA MODEL EVALUATED BY ECOEXERGY

GARCIA VARGAS, S. A.[1] – PEÑA MORENO, R. D.[2] – LINARES GONZÁLEZ, Y. A.[1] – SERKIN, V.[2] – MORALES LARA, L.[3*]

[1]*Posgrado en Ciencias Ambientales, Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla, Edificio IC 8, Ciudad Universitaria, C. P. 72570 Puebla, México*

[2]*Centro de Química, Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla, Av. 4 Sur 104, C. P. 72000 Puebla, México*

[3]*Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla, Av. 4 Sur 104, C. P. 72000 Puebla, México*

[*]*Corresponding author*
e-mail: laura.morales@correo.buap.mx

(Received 24th Feb 2023; accepted 1st Jun 2023)

Abstract. Chiltepin (*Capsicum annuum* var. *glabriusculum*) is a wild plant resource with a wide geographical distribution in Mexico and it is part of life in rural societies in the state of Puebla. The pungency of chiltepin is due to capsaicinoids, especially capsaicin and dihydrocapsaicin, which are of interest to the agricultural industry due to their insecticidal, bactericidal, and fungicidal effects; however, both a phytotoxic and promoter effects, have not been explored in *in-vitro* cultures of *Arabidopsis thaliana*. Therefore, the objective of this research was to evaluate the promoter and phytotoxic effect of ethanolic extracts of chiltepin from the Sierra Norte of Puebla, Mexico, on *Arabidopsis thaliana* crops based on their energy use evaluated by ecoexergy through neural networks. The ethanolic extract with seed (EECS) and without seed (EESS) was obtained by ultrasound-assisted extraction (UAE) of ripe fruits, using 96% ethanol. Capsaicin content was determined by UV/VIS spectrophotometry. EECS, EESS and EtOH extracts were added in different amounts to the MS medium in which 7-day-old *Arabidopsis thaliana* seedlings were grown. The concentrations at which a phytotoxic effect was observed after 120h were 28.37 mg/L and 35.62 mg/L, for EECS and EESS respectively. Exposure to a dose 10 times lower than the EC_{50}, in the case of EECS, showed an increase in the calculated energy.
Keywords: *neural network, Puebla, CE_{50}, Capsicum annuum var. glabriusculum, seedling*

Introduction

Chili (*Capsicum*) is an important element in Mexican culture and food, its role in pre-Hispanic cultures has been widely documented (Long-Solís, 1986) and it is currently among the seven vegetables with the highest production worldwide (Latournerie et al., 2015). Chiltepin (*Capsicum annuum* var. *glabriusculum*) is a wild plant resource with a wide geographical distribution in Mexico (Reyes et al., 2019). In Puebla, Mexico, the cultivation of this food is part of the gastronomy in rural societies (Bañuelos et al., 2008; Morales et al., 2018) who consume it as a seasoning in regional food.

Pungency of chiltepin is due to capsaicinoids, the most abundant being capsaicin (8-methyl-N-vanillyl-6-nonenamide) and dihydrocapsaicin (8-Methyl-N-vanillyl-nonamide) (Stan et al., 2021). In recent years, these compounds have become of interest to the agricultural industry due to their insecticidal, bactericidal, and fungicidal effects (Fayos et al., 2018).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2105_38373848

Because of that, the extracts of carotenoids and phenols present in chiltepin fruits stand out, given that they inhibit the growth of fungi of agricultural importance in Mexico (Rodríguez et al., 2015); however, the effects of ethanolic extracts of chiltepin obtained by ultrasound-assisted extraction (UAE) have not been fully explored, although it is known that some secondary metabolites such as terpenoids, phenolic compounds, phenylpropanoids, stilbenes, alkaloids and saponins present in plant extracts (Dixon, 2001) are easily degraded in soil and do not have toxic effects on mammals, which allows their use in organic and sustainable agriculture systems (Rodríguez et al., 2015).

At the same time, studies are needed that facilitate the estimation and prediction of the effects of the application of said extracts from the energy perspective, which can be quantified with ecoexergy. Ecoexergy is defined as the useful part of the energy (kilojoules) involved in the vital processes of a system and is calculated by multiplying the biomass by its available energy in genetic information (Jørgensen and Mejer, 1977; Jørgensen, 2015). Thus, the exergy reflects the degree of development and growth of a system whose components and configurations always seek to maximize the flow of useful energy and stored exergy (Silow and Mokry, 2010).

Similarly, it can be noted that it is possible to use neural networks as a predictive method that conceives the variations in the input data, which makes it a flexible modeling approach for decision making (Zhang et al., 2020). Thus, the objective of this work is the evaluation of the promoter and phytotoxic effect of ethanolic extracts of chiltepin from the Sierra Norte de Puebla, Mexico, on *Arabidopsis thaliana* crops based on their energy use evaluated by ecoexergy through neural networks.

Target

The objective of this study was to evaluate the promoter and phytotoxic effect of ethanolic extracts from ripe chiltepin fruits in the *Arabidopsis thaliana* model by quantifying ecoexergy.

Materials and methods

Collection of samples and obtaining extracts

Ripe chiltepin fruits were collected and subsequently dried in a conventional oven at 60 °C for 4 h. Then, the material was separated into two samples: one with the fruit and its seeds (EECS) and the other with the fruit but no seeds (EESS). Both samples were crushed in a blender at medium speed until fine powder was obtained, to which 96% ethanol was added. The compounds of both samples were extracted with an ultrasonic bath during a period of 25 minutes at 55 °C and 40 kHz (Barbero et al., 2008). Finally, the obtained extract was filtered twice, the first time to remove larger solids and the second one with the objective of eliminating viruses or bacteria through a 0.22 µm membrane (Lu et al., 2017).

Capsaicin quantification

The capsaicinoid content in the ethanolic extracts of ripe chiltepin fruits, EESS and EECS, was determined by using the standard curve of the pure compound 8-Methyl-N-vanillyl-trans-6-nonenamide (Sigma-Aldrich – 12084) at concentrations of 25, 50, 75 and 100 mg/L by UV absorption spectroscopy at a wavelength of 266 nm (Genesis 10uv-ThermoScientific). This was done from the identification of the maximum absorbance

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2105_38373848

through spectral scanning of the extracts in the region between 230 to 280 nm (Davis et al., 2007; González et al., 2015). The absorbances were processed by logistic regression with the software RCommander (adjusted $R^2 = 0.9762$).

Acute test of extracts on A. thaliana seedlings

7-day-old seedlings grown in MS medium (Phytotechnology with added vitamins) were exposed to concentrations of 27.20, 30.60 and 34.00 mg/L of capsaicin from EESS and 26.17, 29.43 and 32.71 mg/L from EECS in a final volume of 20 mL of culture medium. The reference groups included cultures in the absence of the extracts and one with the same volume of 96% ethanol applied along with the EESS and EECS treatments. All treatments were carried out in triplicate, each with four seedlings and incubating at 25 °C with monitoring at 72 and 120 h.

The mean lethal concentrations for both extracts were established referring to the loss of coloration of the seedlings exposed over 5 days. The data were approached using probit analysis to calculate the EC_{50} from the mortality percentage with respect to the color of the control seedlings (*Table 1*). Similarly, the ethanol extract was included to rule out the possible effects of 96% ethanol, which was used as a mean of extracting the compounds in the fruits.

Chronic test of extracts on A. thaliana seedlings

Chronic exposure was carried out for a period of 20 days at concentrations of 3.40 mg/L and 3.27 mg/L of capsaicin from EESS and EECS, respectively, in a volume of 20 mL of MS culture medium, four seedlings were used in each replicate with 7 days old. Again, the control groups consisted of culture media without ethanolic extracts, as well as the treatment that considered only 96% ethanol. An analysis of variance (ANOVA) type 1 was performed as well as a Tukey post-hoc test to determine significant differences between groups in the R commander program (ver. 4.2.2).

Arabidopsis thaliana ecoexergy calculation

The echoexergy calculation for the seedlings exposed to both extracts, EECS and EESS, was carried out by recording the changes in fresh weight (*Eq.1*) per seedling considering the measurements of the width and length of the leaf, as well as the length of the stem in mm. The above from the use of the following *Equation 2* (Jørgensen, 2005):

$$\begin{aligned} Fresh\,weight = {} & Leaf\ width\,mm\,(-6.5538) \\ & + Leaf\ length\,mm\,(2.86677) \\ & + Stem\ length\,(3.7163) - 13.4245\,* \end{aligned} \qquad \text{(Eq.1)}$$

* Multiple regression model, $R^2 = 0.8297$, P value: 0.0005496, reference this work.

$$Ecoexergy = \Sigma\beta i * Ci \qquad \text{(Eq.2)}$$

where:
βi = The weighting factor of the organism according to the amount of bp contained in its genome,
ci = Concentration of the Carbon component of the dry biomass,
β value for the *Arabidopsis thaliana* model was obtained by using the following equation:

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2105_38373848

$$\beta i = \frac{In\left(20^{-\left(\frac{C_i}{2}\right)\left(\frac{bp}{3}\right)}\right)1}{Ex_{det}} \quad \text{(Eq.3)}$$

where:
$Ci = C$
$bp = 9.8 * 10^8$
$Ex_{det} = 7.43 * 10^5$

The quantification of carbon biomass per seedling was established based on the data collected through the dry weight of the seedlings at 105 °C in an oven (BLUE M) and the use of the value reported by Küstner et al. (2019) for the calculation of carbon content:

$$Dry\ weight = (0.0534\ Fresh\ weight\ g) + (-0.000092885\ Water\ loss\ percentage) + 0.00877 * \quad \text{(Eq.4)}$$

* Linear regression model, R^2 = 0.999, P-value: 0.00707, Reference this work.

$$Ci = Dry\ weight\ [mg] * 0.45 \quad \text{(Eq.5)}$$

The echoexergy data calculated in each treatment were processed in R commander (ver. 4.2.2) using a type 1 analysis of variance (ANOVA) as well as a Tukey test to determine significant differences between groups.

Design of a neural network for the calculation of ecoexergy

The neural network used for the echoexergy calculation was built in the Rstudio ID, the "neuralnet", "MASS", "ROCR", "gmodels", "caret" and "lattice" libraries were used in the network programming whose architecture consisted of a perceptron of 4 information inputs with 3 hidden layers and an output with the average echoexergy recorded at concentrations of 3.27 and 3.40 mg/L in the treatments with EECS, EESS and 7970 mg/L of EtOH. The "Resilient Backpropagation" algorithm, which updates the weights considering the sign of change, was used to activate the network with a total of 1000 iterations.

Results and discussion

Capsaicin quantification in the ethanolic extracts

The wavelength of maximum absorbance for the extracts corresponded to 266 nm (*Figure 1*), it was identified from a spectral scan between 230 and 280 nm, which was used in the quantification of the ethanolic extracts of chiltepin, EESS and EECS.

Thus, the quantification of the capsaicin standard was established at 266 nm and was corroborated with a scan of the standard within the aforementioned interval (*Figure 2*).

It was observed that most of the capsaicinoids presented some absorption in the UV region of the spectrum, between 200 and 350 nm. Therefore, by using the Beer-Lambert law (González et al., 2015) it was possible to estimate the quantity of capsaicin contained in the extracts: 340.06 mg/L for EESS and 327.15 mg/L for EECS.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2105_38373848

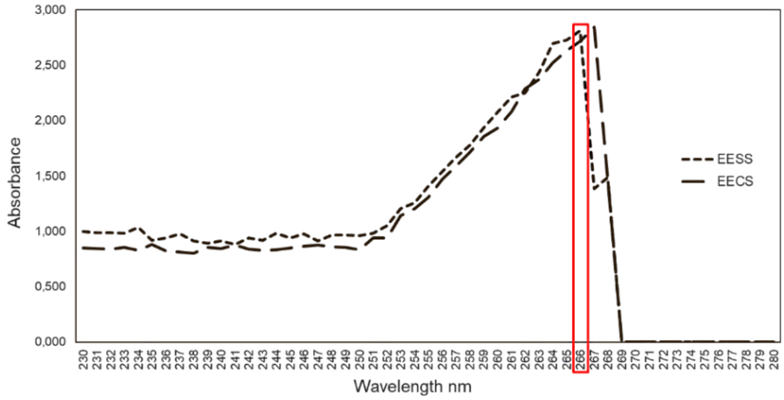

Figure 1. *UV spectra of chiltepin (Capsicum annuum L. var glabriusculum) extracted with ethanol. Spectra obtained at wavelengths between 230 and 280 nm for the EESS and EECS extracts*

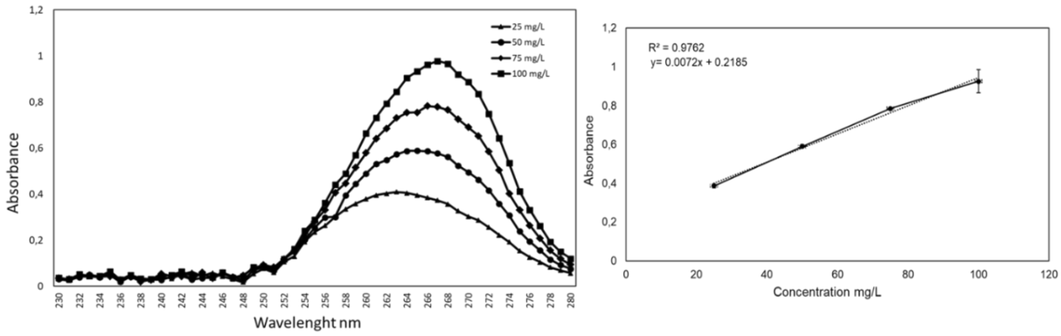

Figure 2. *UV spectra at different concentrations of the capsaicin standard (a). Calibration curve of each capsaicin (b). The equation of the regression line for capsaicin was (y=0.0072x + 0.2185)*

EC_{50} determination

Table 1 summarizes the phytotoxic effect of exposure to ethanolic extracts of chiltepin for 5 days, estimating the mean effective concentration at 72 and 120 h using the probit method. 50% mortality was observed in 7-day-old seedlings in a time of 72 h at a concentration of 39.84 mg/L for EECS and 35.78 mg/L for EESS. It should be noted that prolonging the exposure period (48 additional hours) increased the effectiveness for EECS as compared to EESS. Although there is no information about the long-term effect of high doses of chiltepin extracts on *Arabidopsis thaliana*, capsaicinoids isolated from *Capsicum annuum* L. have been evaluated in *Lactuca sativa* seedlings, concluding that the inhibition is dose-dependent (Wang et al., 2020). Regarding EtOH, concentrations higher than 10^6 mg/L are required to cause 50% mortality.

Table 1. *Herbicidal effect of ethanolic extracts of chiltepin*

Treatment	CE_{50} 72 h (3rd day)	CE_{50} 120 h (5th day)
Ethanolic extract with seed (EECS)	39.84 mg/L ****	28.37 mg/L ***
Ethanolic extract without seed (EESS)	35.78 mg/L***	35.62 mg/L***
Ethanol (EtOH)	6.23 x 10^7 mg/L	3.30 x 10^7 mg/L

Significance code: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2105_38373848

Such a precedent implies that the effective dose for EECS when presented with prolonged exposure is lower compared to EESS (*Figure 3*), which can be attributed to the presence of saponins in chiltepin seeds; it is to be noted that Osorio et al. (2009) associate a phytotoxic effect with these compounds.

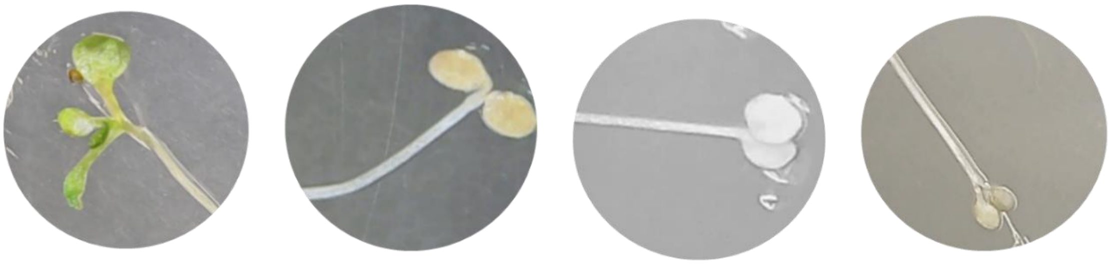

Figure 3. *Phytotoxicity of the ethanolic extracts of chiltepin and solvent used at 120 h on Arabidopsis thaliana*

Calculation of ecoexergy for Arabidopsis thaliana

The effects of the ethanolic extracts of chiltepin were modeled through ecoexergy of *Arabidopsis thaliana*. The quantification of ecoexergy allowed us to know the energy flow and its orientation by combining structural and functional components of *A. thaliana*. The calculation considered the carbon biomass in the seedlings, the βi and the value of the detritus as energy reference (*Table 2*).

Table 2. *Information used for mathematical modeling*

Variable	Formula	Reference
Carbon biomass (g)	Dry weight g * 0.45	Küstner et al., 2019
βi coefficient A. thaliana	81.00 g/m^2	Calculated according to Libralato et al., 2006; Jørgensen et al.,2005; Álvarez et al., 2015.
Detritus reference value	18.7 kJ/g	Jørgensen et al.,1995; Álvarez et al., 2015, Jorgensen & Fath 2004.

Ecoexergy of acute exposure in the A. thaliana model

Acute exposure to EECS and EESS caused loss of color in the leaves of the seedlings due to the high concentrations of compounds with phytotoxic potential, which also had negative effects on their growth and energy (Jorgensen, 1995; Libralato et al., 2006). The reduction in the energy use of the seedlings in the presence of capsaicin (*Figure 4*) was more notable with EECS at the concentration of 35.98 mg/L ($p<0.05$), but not for the treatments with EtOH and EESS ($p > 0.05$); however, a decrease in ecoexergy was observed with respect to the control. The energy decrease is proportional to the loss of biomass derived from the presence of compounds in chiltepin extracts such as tannins, glycosides, terpenoids, flavonoids, saponins, reducing sugars, steroids and resins that are responsible for the inhibitory effects (Rosulu et al., 2022) on seedlings.

Energy loss became evident when comparing the groups subjected to EESS, EECS and EtOH regarding the average ecoexergy recorded in the control group at 72 h

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2105_38373848

(*Figure 5*). A decrease in ecoexergy was observed in the EESS treatment, which contrasts with the assumption of an increase in capsaicin ($p < 0.05$), the same effect was recorded for the EtOH group.

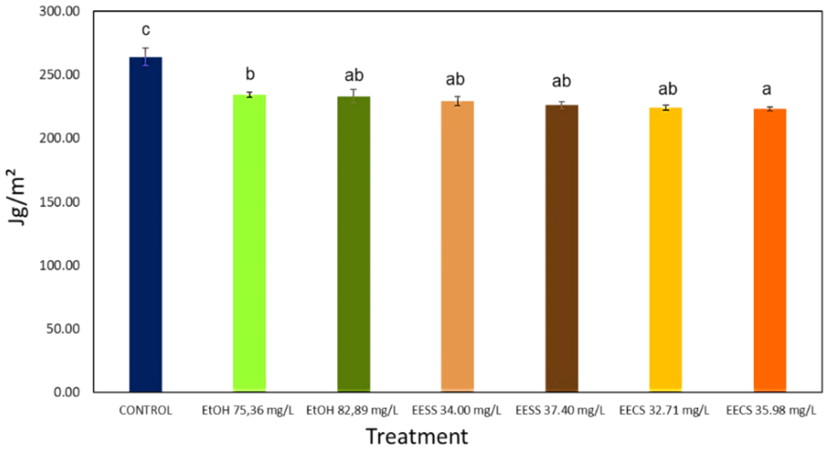

Figure 4. *Ecoexergy of Arabidopsis thaliana exposed to EC_{50} concentrations for 72h. From the fresh weight of seedlings exposed to EESS, EECS and EtOH.*

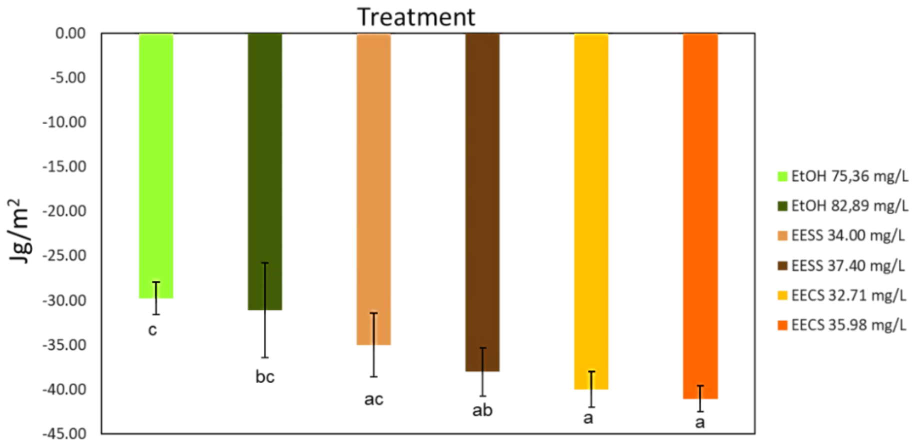

Figure 5. *Difference in ecoexergy in Arabidopsis thaliana due to acute exposure considering the weight of the seedlings at the end of the exposure with EESS, EECS and EtOH*

On the contrary, mortality of 50% of the seedlings was observed in the groups with EECS in a capsaicin interval of 32.71 to 35.98 mg/L, which entails an evident decrease in ecoexergy ($p < 0.05$) regarding the EtOH and EESS treatments. It is noteworthy how the phytotoxic effect considers not only the presence of capsaicin, but also other compounds that are present in the seed of chili peppers (Cervantes-Hernández et al., 2019; Rosulu et al., 2022).

Ecoexergy of A. thaliana on chronic exposure

Exposure to low concentrations of capsaicin in ethanolic extracts of chiltepin on models such as *Arabidopsis thaliana* has not been fully explored, therefore, it was essential to analyze the energy changes that could occur in exposed seedlings based on

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2105_38373848

the calculated fresh weight of the parameters. width and length of the leaf, as well as the length of the stem in mm during 20 days at EESS, EECS and EtOH with four-time cuts at days 5, 10, 15 and 20 (*Figure 6*).

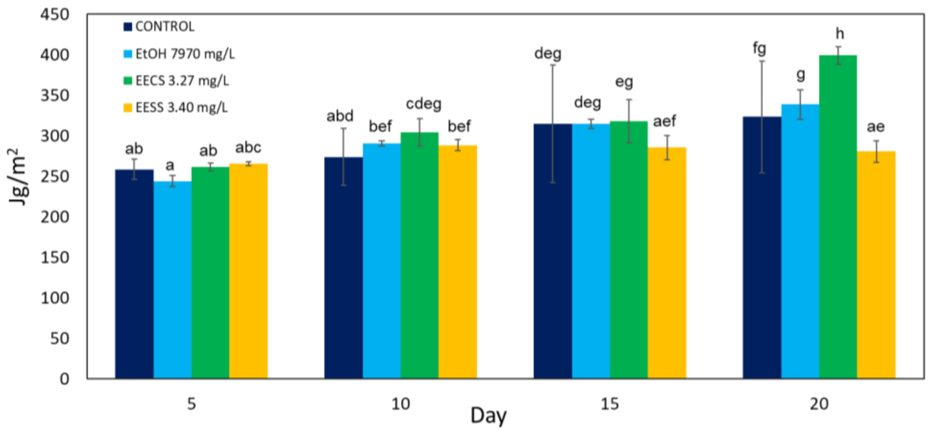

***Figure 6.** Ecoexergy of chronic exposure with EESS, EECS and EtOH in an Arabidopsis thaliana model*

It should be noted that, in the case of EECS, exposure to a dose 10 times lower than that determined in the EC50 showed an increase in the specific energy on days 5, 10 and 20 in the exposed seedlings, at the same time, a positive evolution of the same (*Figure 7*), not so for day 15, which would indicate a process of adaptation of the seedlings to the presence of saponins and terpenoids (López-Muñoz et al., 2019). However, the effects of the treatment with EESS reveal a slight increase on days 5 and 10 ($p > 0.05$), with significance in the energy decrease on days 15 and 20, which can be attributed to the absence of flavonoids, which are responsible for of the antioxidant activity in plants (Vásquez, 2015), these compounds are present in the placenta of Capsicum fruits (Cervantes-Hernández, 2019), a section discarded in the preparation of the EESS extract. Regarding the exposure that only considers the EtOH solvent, a decrease in Jg/m^2 was noted on days 5 and 15 with increases on days 10 and 20, which could indicate adaptation of the seedlings during those periods of time (Chen et al., 2015; Bui et al., 2019).

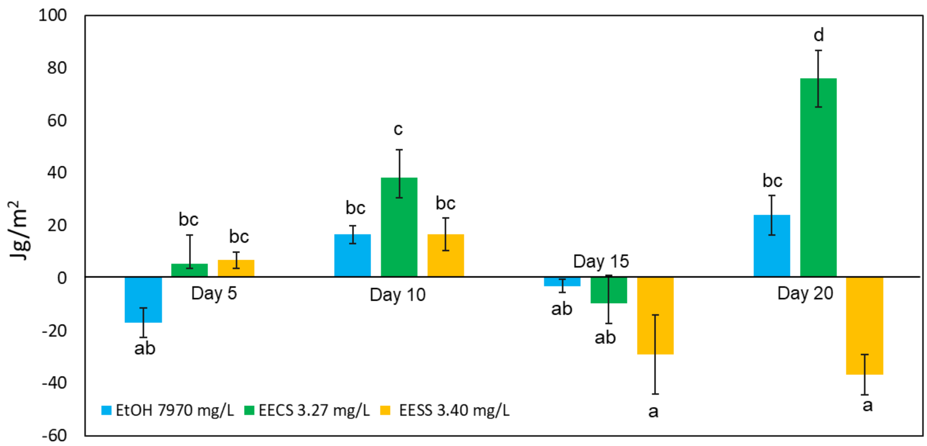

***Figure 7.** Difference in ecoexergy for chronic exposure of Arabidopsis thaliana with EESS, EECS and EtOH*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2105_38373848

Ecoexergy prediction in A. thaliana using neural networks

The construction of a deep learning neural network with a multilayer structure (*Figure 8*) and supervised learning algorithms (Johnson et al., 2017; Horie, 2019) facilitated the admission of input variables as signals between the hidden layers, which improved the prediction of ecoexergy (Geetha and Thilagam, 2021). Thus, the variables used in this study were: Exposure time, Extract concentration, Fresh weight (g), Biomass (g) and, as output layer, the Ecoexergy value (Jg/m^2).

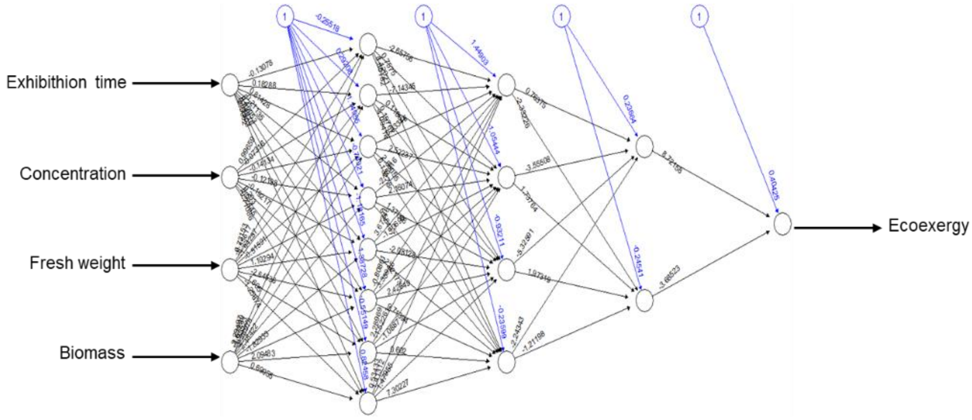

Figure 8. *Architecture of the neural network for the prediction of ecoexergy in A. thaliana that was chronically exposed to EESS, EECS and EtOH*

The prediction of the ecoexergy values (at 98% precision) for *Arabidopsis thaliana* in treatments at low concentrations of ethanolic extracts of chiltepin was calculated using "R-prop+" as an activation equation, which is available in the neuralnet library at Rstudio.

The ecoexergy values predicted by means of the neural network were close to those quantified from the fresh weight of the seedlings (*Figure 9*), which highlights the importance of prior training of the network in the face of changes in the input variables (Exposure time, Concentration and Fresh Weight), in order for the predictions to improve substantially.

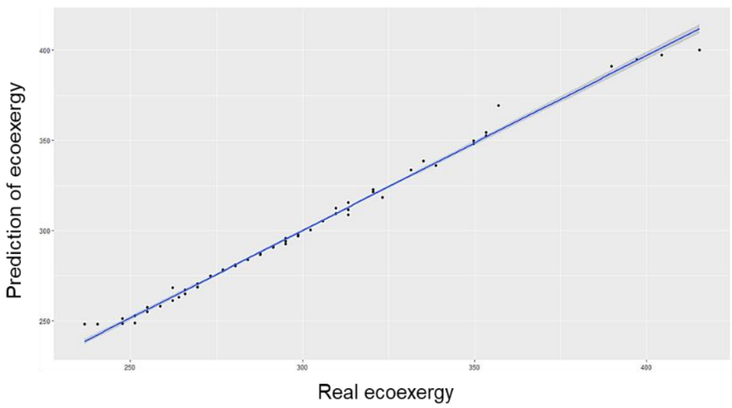

Figure 9. *Actual ecoexergy vs Calculated ecoexergy by the neural network for A. thaliana chronically exposed to EESS, EECS and EtOH*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2105_38373848

Ecoexergy values produced by the neural network were weighted for their analysis, positive evolution was coded with the numerical value 1, while negative evolutions were characterized with 0. Ecoexergy data for the treatment with EECS on day 20 registered an output of 1 with the consequent activation of the nodes of the first layer, allowing the data to be passed to the subsequent layer in the network. Values above 300 Jg/m^2 presented a positive development for treatment with EECS, which indicated the hierarchy of variables in the ecoexergy calculation (*Figure 10*): Biomass > Fresh weight > Exposure time > Concentration in the treatments (EECS, EESS and EtOH) and control.

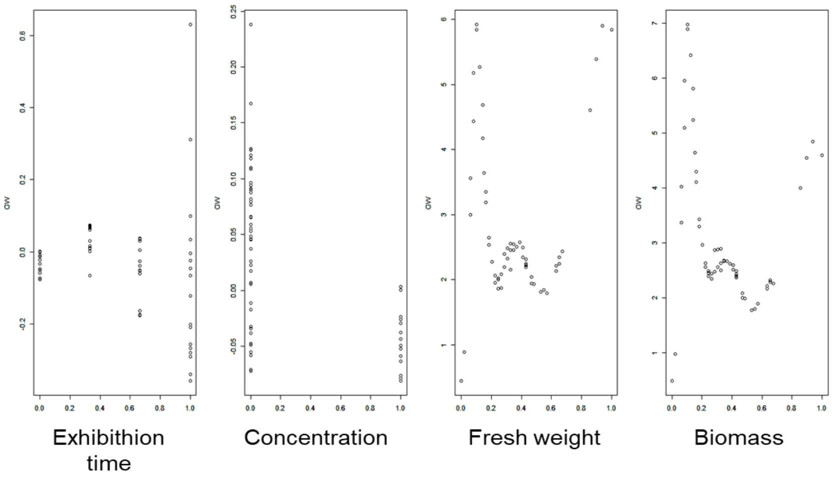

Figure 10. *Weight of the input variables in the multilayer neural network*

Thus, the contribution of the variable Biomass in the total value of ecoexergy stood out, given that its importance was greater than that of the remaining variables since the exergy residing in each treated seedling was obtained from the aforementioned value. Therefore, the design of this neural network preserved the ecoexergy estimation principle by considering biomass and fresh weight as important parts of the information transferred through the nodes in the hidden layers.

Conclusions

Ethanolic extracts obtained from ripe chiltepin fruits from the Sierra Norte de Puebla showed a phytotoxic and promoter effect. The phytotoxicity evaluated in *Arabidopsis thaliana* in the short term was better with EESS treatments, while the EECS reflected a decrease in ecoexergy as a result of greater phytotoxicity in the long term.

By the same token, the decrease in the volume of the extracts had a beneficial effect on *A. thaliana* by registering increases in ecoexergy for culture media with EECS. In-vitro tests for EECS and EESS elucidate a panorama of benefits that can be obtained from mixing organic compounds extracted from ripe chiltepin fruits, which evaluated with holistic parameters, such as ecoexergy, clarify the possible effects in a microsystem which in turn can be complemented by using modeling tools, such as neural networks, to predict long-term effects.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2105_38373848

REFERENCES

[1] Bañuelos, N., Salido, P. L., Gardea, A. (2008): Etnobotánica del chiltepin. Pequeño gran señor de la cultura de los Sonorenses. – Centro de Investigación y Desarrollo A.C. 16(32): 7-30.
[2] Barbero, G. F., Liazid, A., Palma, M., Barroso, C. G. (2008): Ultrasound-assisted extraction of capsaicinoids from peppers. – Talanta 75(5): 1332-1337.
[3] Bui, L. T., Novi, G., Lombardi, L., Iannuzzi, C., Rossi, J., Santaniello, A., Mensuali, A., Corbineau, F., Giuntoli, B., Perata, P., Zaffagnini, M., Licausi, F. (2019): Conservation of ethanol fermentation and its regulation in land plants. – Journal of Experimental Botany 70(6): 1815-1827.
[4] Cervantes-Hernández, F., Alcalá-González, P., Martínez, O., Ordaz-Ortiz, J. J. (2019): Placenta, pericarp, and seeds of tabasco chili pepper fruits show a contrasting diversity of bioactive metabolites. – Metabolites 9(10): 206.
[5] Chen, L., Liao, B., Qi, H., Xie, L. J., Huang, L., Tan, W. J., Zhai, N., Yuan, L.-B., Zhou, Y., Yu, L.-J., Chen, Q.-F., Shu, W., Xiao, S. (2015): Autophagy contributes to regulation of the hypoxia response during submergence in Arabidopsis thaliana. – Autophagy 11(12): 2233-2246.
[6] Davis, C. B., Markey, C. E., Busch, M. A., Busch, K. W. (2007): Determination of capsaicinoids in habanero peppers by chemometric analysis of UV spectral data. – Journal of agricultural and food chemistry 55(15): 5925-5933.
[7] Dixon, R. A. (2001): Natural products and plant disease resistance. – Nature 411(6839): 843-847.
[8] Fayos Avellán, O., Martínez, O., Ochoa Alejo, N., Savirón, M., Orduna, J., Garcés Claver, A. (2018): Evolución del contenido de capsinoides y capsaicinoides durante la maduración de los frutos de 'chiltepin' *C. annuum* L. var. glabriusculum. – IX Congreso de Mejora Genética de Plantas 2018. Murcia, 18 - 20 de septiembre de 2018.
[9] Geetha, R., Thilagam, T. (2021): A review on the effectiveness of machine learning and deep learning algorithms for cyber security. – Archives of Computational Methods in Engineering 28(4): 2861-2879.
[10] González-Zamora, A., Sierra-Campos, E., Pérez-Morales, R., Vázquez-Vázquez, C., Gallegos-Robles, M. A., López-Martínez, J. D., García-Hernández, J. L. (2015): Measurement of capsaicinoids in chiltepin hot pepper: a comparison study between spectrophotometric method and high-performance liquid chromatography analysis. – Journal of Chemistry 2015: 709150.
[11] Horie, T. (2019): Global warming and rice production in Asia: Modeling, impact prediction and adaptation. – Proceedings of the Japan Academy, Series B 95(6): 211-245.
[12] Johnson, M., Kang, M. J., Lawson, T. (2017): Stock price reaction to data breaches. – Journal of Finance Issues 16(2): 1-13.
[13] Jørgensen, S. E., Mejer, H. (1977): Ecological buffer capacity. – Ecological Modelling 3(1): 39-61.
[14] Jørgensen, S. E., Nielsen, S. N., Mejer, H. (1995): Emergy, environ, exergy and ecological modelling. – Ecological modelling 77(2-3): 99-109.
[15] Jørgensen, S. E., Fath, B. D. (2004): Application of thermodynamic principles in ecology. – Ecological complexity 1(4): 267-280.
[16] Jørgensen, S. E., Ladegaard, N., Debeljak, M., Marques, J. C. (2005): Calculations of exergy for organisms. – Ecological Modelling 185(2-4): 165-175.
[17] Jørgensen, S. E. (2015): New method to calculate the work energy of information and organisms. – Ecological modelling 295: 18-20.
[18] Küstner, T., Armanious, K., Yang, J., Yang, B., Schick, F., Gatidis, S. (2019): Retrospective correction of motion-affected MR images using deep learning frameworks. – Magnetic resonance in medicine 82(4): 1527-1540.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2105_38373848

[19] Latournerie-Moreno, L., López-Vázquez, J. S., Castañón-Nájera, G., Mijangos-Cortéz, J. O., Espadas-Villamil, G., Pérez-Gutiérrez, A., Ruiz Sánchez, E. (2015): Evaluación agronómica de chile habanero (Capsicum Chinense Jacq.). – Agroproductividad 8: 24-29.
[20] Libralato, S., Torricelli, P., Pranovi, F. (2006): Exergy as ecosystem indicator: An application to the recovery process of marine benthic communities. – Ecological Modelling 192(3-4): 571-585.
[21] Long-Solís, J. (1986): Capsicum y cultura: la historia de chilli. – Fondo de Cultura Económica, México.
[22] López-Muñoz, N. R., Romero-Bastidas, M., Arce-Amézquita, P. M., Hernández-Rubio, J. S. (2019): Actividad antifúngica de antioxidantes derivados de cuatro cultivares de Capsicum spp. contra hongos fitopatógenos. – Ecosistemas y recursos agropecuarios 6(18): 487-498.
[23] Lu, M., Ho, C. T., Huang, Q. (2017): Extraction, bioavailability, and bioefficacy of capsaicinoids. – Journal of food and drug analysis 25(1): 27-36.
[24] Morales-Guzmán, V., Morales-Calva, E., Gallardo-Sandoval, A., Ortega-Rangel, L. (2018): Cultivo de chile piquín (*Capsicum annum*) y evaluación de calidad postcosecha en Xicotepec de Juárez.
[25] Osorio Salazar, L., Valverde, F. A., Bonilla Correa, C. R., Sánchez Orozco, M. S., Mier Barona, C. E. (2009): Evaluación de extractos de fique, coquito, sorgo y ruda como posibles bio-herbicidas. – Acta Agronómica 58(2): 103-107.
[26] Reyes-Acosta, D. J., Álvarez-Parrilla, E., Jiménez-Alvarado, R., Campos-Montiel, R. G., Hernández-Fuentes, A. D. (2019): Actividad antioxidante de los extractos de Chiltepin (*Capsicum annum*) cultivados bajo redes de colores. – Boletín de Ciencias Agropecuarias del ICAP 5(10): 1-5.
[27] Rodriguez-Maturino, A., Troncoso-Rojas, R., Sánchez-Estrada, A., González-Mendoza, D., Ruiz-Sanchez, E., Zamora-Bustillos, R., Cecena-Duran, C., Grimaldo-Juarez, O., Aviles-Marin, M. (2015): Antifungal effect of phenolic and carotenoids extracts from chiltepin (Capsicum annum var. glabriusculum) on Alternaria alternata and Fusarium oxysporum. – Revista Argentina de Microbiología 47(1): 72-77.
[28] Rosulu, H., Oni, M., Ofuya, T., Adebayo, R. (2022): Bioefficacy of chilli pepper *Capsicum frutescens* (L.) and it's intercropping on the growth, yield, and insect pest management of cowpea *Vigna unguiculata* (L.) walp in the rain forest area of Nigeria. – Journal of Global Agriculture and Ecology 13(2): 7-18.
[29] Silow, E. A., Mokry, A. V. (2010): Exergy as a tool for ecosystem health assessment. – Entropy 12(4): 902-925.
[30] Stan, T., Munteanu, N., Teliban, G. C., Cojocaru, A., Stoleru, V. (2021): Fertilization Management Improves the Yield and Capsaicinoid Content of Chili Peppers. – Agriculture 11(2): 181.
[31] Vásquez, M. R. S. (2015): Estudio fitoquímico y cuantificación de flavonoides totales de las hojas de *Piper peltatum* L. y *Piper aduncum* L. procedentes de la región Amazonas. – In Crescendo 6(1): 33-43.
[32] Wang, R., Liu, Y., Sun, S., Si, Y., Liu, X., Liu, X., Zhang, S., Wang, W. (2020): Capsaicinoids from hot pepper (*Capsicum annuum* L.) and their phytotoxic effect on seedling growth of lettuce (*Lactuca sativa* L.). – Natural product research 34(11): 1597-1601.
[33] Zhang, C., Chen, Y., Xu, B., Xue, Y., Ren, Y. (2020): Improving prediction of rare species' distribution from community data. – Scientific reports 10(1): 1-9.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2105_38373848