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WALNUT (JUGLANS REGIA): A REVIEW OF PHYTOSANITARY PROPERTIES AND THEIRS MATHEMATICAL MODELING

BY

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Abstract. Walnut (Juglans regia L.) is the most widespread walnut in the world. All parts of the plant are important: bark, leaves, dried and green peel of the fruit, septum, core. The benefits of walnuts are due to the presence of phytochemicals such as flavonoids, carotenoids, alkaloids, polyphenols, etc. In addition to the many benefits that walnut has on our health (antibacterial, antioxidant), it also has important phytosanitary and insecticidal properties. Walnuts can be used because of their plant-friendly properties in the form of biopesticides that are safe and can be a viable, inexpensive and cleaner alternative to synthetic products that can be harmful to the environment. This review paper seeks to bring to the fore the available literature on Juglans regia directed on the properties, antifungals and insecticides with action on plants and antibacterials with action on the human body, as well as mathematical models regarding the multiplication of microorganisms.

Keywords: walnut, antifungal, insecticide, antibacterial, mathematical modeling.

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1. Introduction

Walnut is a crop of great economic interest to the food industry. The tasty part of the fruit (core) is eaten fresh or fried, alone or mixed with other edible products. It is popular all over the world and appreciated for its nutrition and health. The core is a dense nutrient mainly due to the content of fats, proteins, vitamins, flavonoids, phenolic acids, polyphenols and minerals.

Also, the other components of the plant have a variety of phytosanitary properties with action on other plants (antifungals and insecticides) or have benefits on the human body (antibacterial, antifungal).

Phytosanitary properties can be processed mathematically. Mathematical modeling is a useful tool in data processing.

2. Phytosanitary properties

2.1. Antifungal properties

Pathogenic fungi are responsible for crop damage in both pre-harvest and post-harvest stages. Attempts are being made to replace fungicidal treatments that use synthetic chemicals that are polluting with non-toxic plant extracts that are environmentally friendly.

The antifungal activity of walnut extracts has been studied for fungi that affect both crops and vegetables and fruits. The antifungal properties have been observed especially in extracts obtained from walnut bark and from the green shell of the walnut fruit. Walnut extracts have different effects on mushrooms, depending on the section of plant material used and the type of extraction solvent used. In the case of walnut bark extracts, methanolic extract showed significant activity against *Aspergillus niger*, chloroform extract inhibited *Trichoderma virens* and *Fusarium solani*, and acetone extract significantly inhibited the growth of *Alternalia alternata*.

The antifungal activity was performed by the method of diffusion on the disc at concentrations of 100, 200 and 300 $\mu g \cdot mL^{-1}$ / disc of extracts (Upadhyay *et al.*, 2010a). 10% (w / v) methanolic and chloroform extracts from the bark completely inhibited the mycelial growth (growth inhibition (%) = 100%) of the *Geotrichum candidum* fungus that grows on citrus fruits (Ameziane *et al.*, 2007).

Studies have shown that walnut shell extracts contain a substance called Juglone. In this case, bark extracts dissolved in acetone with concentrations of 25.0 were used 10.0; 5.0; 2.5; 1.0 and 0.25 $mg \cdot mL^{-1}$ and Juglone solutions with concentrations from 0.05 to 4 $mg \cdot mL^{-1}$. Solutions with the highest concentrations had results in inhibiting the growth of the mycelium of *Fusarium culmorum, Alternaria alternata, Rhizoctonia solani, Phytophthora infestans* and

Botrytis cinerea. Juglone is not the only one responsible for inhibiting the growth of fungi. *In vitro* results confirmed that phenolic derivatives increase the antifungal activity of Juglone. The inhibitory activity for *A. alternata* is 45% and was determined by measuring the diameter of the inhibitory area and expressed as a percentage using the following equation: Inhibitory activity (%) = [(C - T) x C^{-1}] x 100, where: C- the diameter of the mycelium colony on the control plate (mm); T - diameter of the mycelium colony on the treatment plate (mm) (Wianowska *et al.*, 2016). In addition, the antifungal activity of Juglone has been observed in *Penicillium spp., Aspergilluus spp., Hansenula spp.* and *Saccharomyces carlsbergensis* (brewer's yeast) (Wu *et al.*, 2009; Yakubovskaya *et al.*, 2009).

Walnut extract has a small effect on *Rhizoctonia solani* fungus, which affects the sheath of rice, the relative size of the inhibition being 2.0 mm at a concentration of 1000 ppm extract (Sehajpal *et al.*, 2009).

2.2. Insecticidal properties

Plant extracts are increasingly being used as an alternative to control crop pests. This strategy has no negative consequences for our environment and our health. Walnut extracts also have insecticidal properties. Such effects have been observed in particular in extracts obtained from walnut leaves. Rice weevil (*Sitophilus oryzae*) can be controlled by using walnut leaves as a pesticide. On different days following treatment, contact toxicity revealed that the greatest dose of walnuts (0.1 g·mL^{-1}) was the most effective (66.66%) against rice crust (Sadeghnezhad *et al.*, 2020). The aqueous extract of bedding from walnut leaves with a concentration of 5% caused a mortality of 100% in the nematode species *Heterorhabditis bacteriophora, Steinernema carpocapsae, Steinernema feltiae, Steinernema kraussei* and *Phasmarhabditis hermaphrodita* (Petrikovszki *et al.*, 2019). Walnut extract also discourages the oviposition of the tomato moth (*Tuta absoluta*), with a deterrent effect on egg growth of 79.94% at the highest concentration (20%) (Erdogan, 2019) and has a greater insecticidal action on the larvae *Trichoferus griseus* than the synthetic insecticide Tanalith C (Civelek and Çolak, 2008). At the same time, walnut leaf extracts are ineffective against bean weevil (*Acanthoscelidesobtectus*) (Zlatko *et al.*, 2007).

Juglone, the bioactive compound in walnut extract, in addition to its antifungal properties, also has toxic effects on phytophagous insects, such as: melon or cotton aphid, pumpkin, pepper and tomatoes (*Aphis gossypii Glover*), large wax moth (*Galleria mellonella*), tobacco moth *(Manduca sexta*), cabbage moth (*Trichoplusia ni*), corn moth (*Helicoverpa armigera*), red mite (*Tetranychus urticae*), cabbage butterfly (*Pieris rapae*), vinegar mosquito (*Drosophila melanogaster*), mosquito of yellow fever (*Aedes aegypti*). Juglone acts on the development time of the larvae, decreases the weight of the stern and adults and decreases the number of eggs (Islam and Widhalm, 2020).

2.3. Antibacterial properties

Studies have shown that all parts of the *J. regia* plant (bark, leaves, dry skin and green fruit peel, septum, core) have antibacterial properties.

The antibacterial property of *J. regia* green bark extracts was determined by the method of diffusion on the disc (inhibition zone) and they show good activity against all bacterial *species E. coli, B. subtilis, K. aerogenosa* and *S. aureus* (Pardeep *et al.*, 2013). In a recent study, Juglone was shown to strongly inhibit the three key *Helicobacter pylori* enzymes, cystathionine γ-synthase (HpCGS), malonyl-CoA-acyl transacylase carrier protein (HpFabD) and β-hydroxy acyl-ACP dehydrase (HpFab) with half the values of the maximum inhibitory concentration of 7.0 ± 0.7; 20 ± 1 and 30 ± 4 μmol·L^{-1}, respectively (Jaiswal and Tailang, 2017). The antibacterial properties are improved if the dried walnut shell is modified with silver nanoparticles (AgNP) (Mohan and Panneerselvam, 2021). Antibacterial activity also depends on the extraction solvent. The lowest concentration (5 mg·mL^{-1}) of ethanolic green bark extract was a high antibacterial potential for *Staphylococcus aureus*. The 10 mg·mL^{-1} inhibitory concentration was effective against a Gram-negative species (*E. coli*) and a gram-positive species (*L. monocytogenes*). In contrast, the extract was not able to inhibit *P. aeruginosa* (MIC > 20 mg·mL^{-1}) (Vieira *et al.*, 2020). The 5% $NaHCO_3$ extract showed the best antimicrobial activity. At a concentration of 5 mg·mL^{-1}, the inhibition rates of the tested bacteria (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli*) were 85.31-90.26% (Jahanban-Esfahlan and Amarowicz, 2018). Aqueous extracts inhibited *S. aureus* at the lowest concentration (0.1 mg·mL^{-1}) and a Gram-positive bacteria (*P. aeruginosa*) at the highest concentration (100 mg·mL^{-1}) (Oliveira *et al.*, 2008). Walnut bark and leaf extracts also have an antibacterial effect against tooth decay *Streptococcus mutans* (Abdullah *et al.*, 2020).

Walnut leaf extracts also have antibacterial properties. The antibacterial activity of the hydromethanolic extract for *Staphylococcus aureus* in cow's milk has been shown to be effective (Gomes *et al.*, 2019). An increase in the antimicrobial effect on *Escherichia coli, Pseudomonas aeruginosa* and *Acinetobacter baumannii* is observed if the walnut leaf extract is mixed with ZnO nanoparticles by the green method (Darvishi *et al.*, 2019). Depending on the concentration of the extract (less than 5 mg·mL^{-1}) and the type of extraction solvent, an antibacterial potential with preferential action against Gram-positive bacteria such as *Enterococcus faecalis* and *Listeria monocytogenes* (Vieira *et al.*, 2019) *Staphylococcus epidermidis, Bacillus subtilis, Staphylococcus aureus* (Manzoor *et al.*, 2012), *Bacillus cereus* (Pereira *et al.*, 2007), for ethanolic extracts the inhibitory effect is observed in *Staphylococcus epidermidis* (Nicu *et al.*, 2018), and for methanolic extracts it is observed in *Salmonella arizonae* (MIC = 1.25 mg·mL^{-1}) (Jablia *et al.*, 2017). One study evaluated the inhibitory effects (antibacterial and antibiofilm) of leaf extract on the formation of biofilm

by *P. aeruginosa.* Antibacterial activity was studied using the microtiter plate method and it was determined that the aqueous extract had a better inhibitory activity on planktonic growth, and the methanol extract was more effective on inhibiting *P. aeruginosa* biofilm (Dolatabadia *et al.*, 2018).

Walnut root extract has a strong antibacterial effect against both Gram-positive (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Proteus mirabilis*) bacteria (Huo *et al.*, 2020).

Walnut kernel and film extracts inhibit Gram-positive strains (*E. faecium, E. coli, K. pneumoniae, P. aeruginosa*) (D'Angeli *et al.*, 2021) and coagulase-negative staphylococci (MIC 3.60 - 461.75 $\mu g \cdot mL^{-1}$) (Acquaviva *et al.*, 2019). The alcoholic extract of walnut kernel has a strong antimicrobial effect on Gram-positive bacteria (*Proteus mirabilis, Streptococcus pyogenes, Staphylococcus aureus,*) and was completely ineffective at the concentration tested against Gram-negative bacteria (*Pseudomonas aeruginosa*) (Duda-Seiman *et al.*, 2017). In contrast, aqueous extracts inhibit Gram-positive bacteria (*B. subtilis, B. cereus and S. aureus*) at very low concentrations (0.1-1 $mg \cdot mL^{-1}$) and Gram-negative bacteria (*P. aeruginosa, K. Pneumoniae, E. coli*) at higher concentrations (10-100 $mg \cdot mL^{-1}$) (Pereira *et al.*, 2008).

Septum alcoholic extracts have been demonstrated to have a powerful antibacterial action against Gram-positive bacteria (*S. epidermidis, S. aureus E. faecalis* and *E. faecium*) and less inhibited Gram-negative strains (*E. coli, K. pneumoniae, P. aeruginosa* and *P. mirabilis*) at the same extract concentration (8.75 - 140 $\mu g \cdot mL^{-1}$) (Genovese *et al.*, 2020). After testing the antimicrobial activity of intact and oxidized tannins isolated from walnut septum, it was determined that the oxidation of tannin extract led to increased antibacterial activity for *E. amylovora* and *E. carotovora* bacteria (Lupaşcu *et al.*, 2012).

Walnut bark extracts have been studied for their antibacterial properties. Several types of solvents were used at different concentrations. Ethyl acetate extract showed a high degree of antimicrobial activity for *Streptococcus mutans* and *Streptococcus salivarius* (Kheddouma *et al.*, 2021), the alcoholic extract seems to be more effective for *Staphylococcus aureus* and less for *Salmonella typhi* (Gram-negative bacteria) (Jafer *et al.*, 2020), the aqueous extract (100 µg - 400 $\mu g \cdot mL^{-1}$ / disc) had a significant inhibitory effect on the growth of oral microbial flora compared to the acetone extract (Aldawood *et al.*, 2017; Mutha *et al.*, 2015), the chloroform extract showed *in vitro* antimicrobial activity for microorganisms in dental caries (*Streptococcus mutans, Streptococcus sobrinus, Actinomyces viscosus*) (Nancy *et al.*, 2011), as well as for *Staphylococcus aureus, Esherichia coli* and *Pseudomonas aeruginosa* (Alkhawajah, 1997), and alcoholic extracts, with acetone and benzene with a minimum inhibitory concentration of 50 $\mu g \cdot mL^{-1}$ to 300 $\mu g \cdot mL^{-1}$ inhibits Gram-positive microorganisms (*Bacillus subtilis, Staphylococcus aureus*) (Upadhyay *et al.*, 2010b).

3. Mathematical modeling of phytosanitary properties

3.1. General consideration

Several mathematical methods can be used to model the multiplication of microorganisms. For simplicity, was use variability during inactivation of individual cell to calculate stochastic inactivation of individual cells in a bacterial population. Survival or death is assumed to be the bacterial condition. Bacterial inactivation is independent of other events due to the structure of exponential distributions Weibull. To calculate the stochastic inactivation of a bacterial population, the variability of inactivation times of the initial number of cells and individual cells was studied. The study consists to calculate the probability that a population will contain survivors through a process of stochastic inactivation, rather than the conventional estimation of the D value.

N_0 is the number of bacterial cells at the start of the experiment and $N(t)$ is the number of survivors at time t. The following is how a cell population is inactivated:

$$N(t) = N_0 e^{-\lambda t} \tag{1}$$

where λ is a rate parameter of the exponential distribution. The probability density of the exponential distribution $f(t)$ is given by:

$$f(t) = \lambda e^{-\lambda t} \tag{2}$$

Was calculated a single-cell drop in a population of n ($n \in$ N). The n cells become $n - 1$ cells after one cell inactivation. Population failure rate of n identical individuals was $n\lambda$. The timing of a single-cell decline in a population of n cells can be expressed using Eq. (3):

$$f_{n,n-1}(t) = n\lambda e^{-n\lambda t} \tag{3}$$

where $f_{n,\,n-1}(t)$ is the probability density distribution of the timing of a single-cell decrease in a population of n cells.

The convolution of exponential distributions has been calculated as follows $X_1, \ldots, X_k$ was independent random variables representing the exponential distribution with parameter β_i (i = 1, 2, . . ., k). The sum of multiple variables of exponential distributions ($S_k = X_1 + \ldots + X_k$) was provided by the convolution of the exponential distributions (Akkouchi, 2008; Kulkarni, 2016):

$$f_{S_k}(t) = \sum_{i=1}^{k} \frac{\beta_1 \ldots \beta_k}{\prod_{\substack{j=1 \\ j\neq 1}}^{k} (\beta_j - \beta_i)} exp(-t\beta_i) \tag{4}$$

where $n\lambda \ldots N_0\lambda$ have been substituted for $\beta_1 \ldots \beta_k$.

The stochastic calculations of the time required for a certain decrease in the number of cells is show in Figs. 1 and 2.

The time required for N_0 surviving cells to become $n-1$ surviving cells is:

$$f_{N_0,n-1}(t) = n\binom{N_0}{n}\lambda\sum_{i=n}^{N_0}(-1)^{i-n}\binom{N_0-n}{i-n}e^{-\lambda it} \tag{5}$$

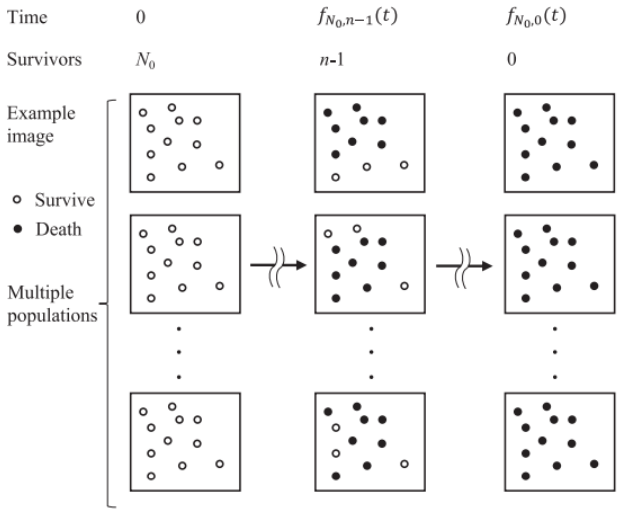

Fig. 1 – A schematic view of stochastic bacterial inactivation (Koyama *et al*., 2019).

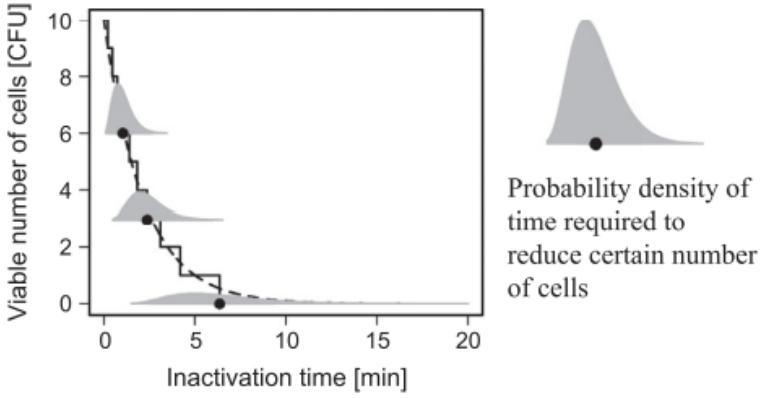

Fig. 2 – The changes in probability density of time required to reduce certain number of cells with λ = 0.2 × log10 and N_0 = 10 cells (Koyama *et al.*, 2019).

The time to inactivation of N_0 cells is therefore defined using Eq. (5):

$$f_{N_0,0}(t) = N_0\lambda \sum_{i=1}^{N_0} (-1)^{i-1} \binom{N_0 - 1}{i - 1} e^{-\lambda it} \tag{6}$$

Using Eq. (6), the probability of a population is:

$$P_{stochastic1}(t) = 1 - \int_0^t f_{N_0,0}(t)dt \tag{7}$$

where $P_{stochastic1}(t)$ is the probability that a population will contain survivors calculated by the time required for a certain reduction in the number of cells.

From Eq. (5), the stochastic bacterial inactivation is a discrete process like (Fig. 3).

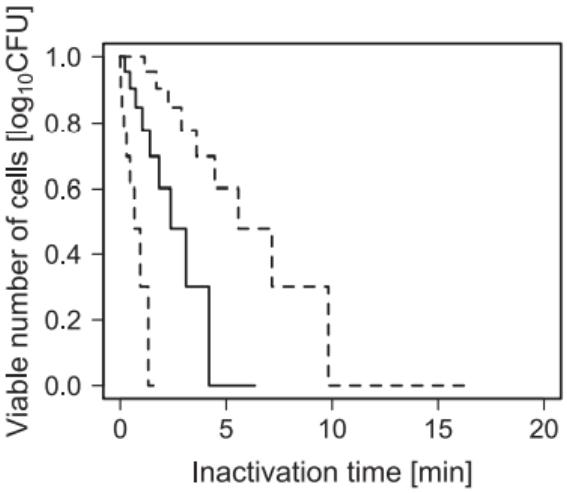

Fig. 3 – Stochastic inactivation model of linear model with λ = 0.2 × log10 and N_0 = 10 cells (Koyama *et al.*, 2019).

From Eq. (7), the probability of a population containing survivors is like in (Fig. 4). The inactivation is described with D-value:

$$N(t) = N_0 10^{-\frac{t}{D}} \tag{8}$$

where D is decimal reduction time (D-value). The probability of a population preserving survivors is characterized by the D-value as:

$$P_{D-value}(t) = N_0 10^{-\frac{t}{D}} \quad (N(t) < 1)$$
$$P_{D-value}(t) = 1 \quad (N(t) \geq 1) \tag{9}$$

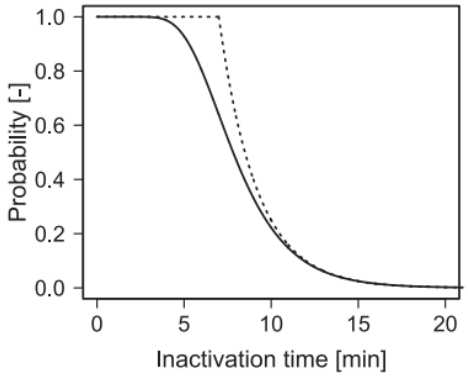

Fig. 4 – Probability of a population containing survivors which initial cell is N_0 = 10 (Koyama *et al.*, 2019).

where D and $P_{D-value}(t)$ are the D-value and the corresponding probability of a population containing survivors, respectively.
The probability that a population will have survivors is also calculated directly from the value of D. The Weibull model calculates stochastic changes in the number of survivors. Model can be written as:

$$N(t) = N_0 e^{-bt^m} \tag{10}$$

where *b* and *m* is the rate and shape parameters of the Weibull distribution (Fig. 5)

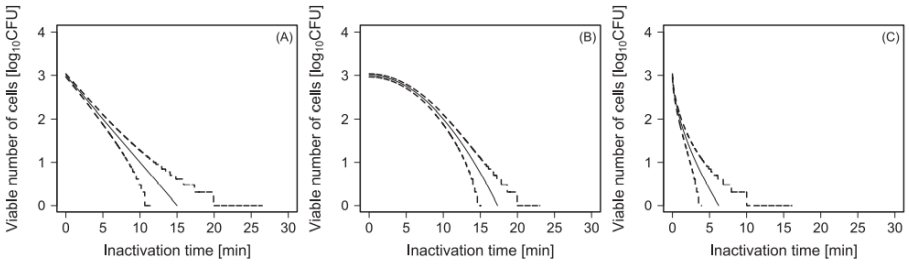

Fig. 5 – Stochastic inactivation model with initial cells $N_0 = 10^3$ (Koyama *et al.*, 2019).

The kinetic model ((Eq. (10)) gives the mean value as follows:

$$N_s(t) \sim Poisson\big(N(t)\big)$$
$$P(X = k) = \frac{N(t)^k exp\big(-N(t)\big)}{k!} \tag{11}$$

where $N_s(t)$ and $P(X = k)$ are the number of survivors at time t and the probability that the number of survivors is equal to k.

An overview of the calculations presented above (Eq. (11)). To calculate the death probability was substitute $k = 0$ in Eq. (11).

The probability of a population containing survivors is calculated as:

$$P_{stochastic2}(t) = 1 - exp\big(-N(t)\big) \tag{12}$$

where $P_{stochastic2}(t)$ is the probability of a population containing survivors calculated by the stochastic change in the number of survivors.

The probability of a population containing survivors can be calculated using the inactivation kinetics (Eq. (10)):

$$P_{kinetic}(t) = N_0 e^{-bt^m} \quad (N(t) < 1)$$
$$P_{kinetic}(t) = 1 \quad (N(t) \geq 1) \tag{13}$$

where $P_{kinetic}(t)$ is the probability of a population (Fig. 6 and Fig. 7)

The difference in probability of a population containing survivors is calculate with $\{(P_{kinetic}(t) - P_{stochastic2}(t)) / P_{kinetic}(t)\}$

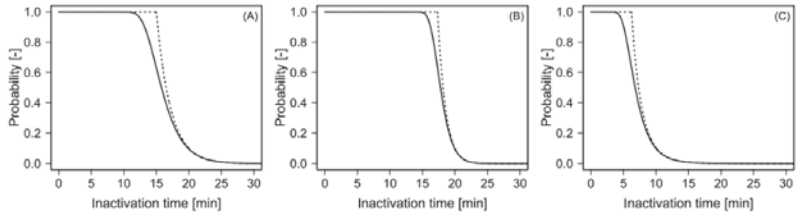

Fig. 6 – Probability of a population containing survivors which initial cell is $N_0 = 10^3$ (Koyama *et al.*, 2019).

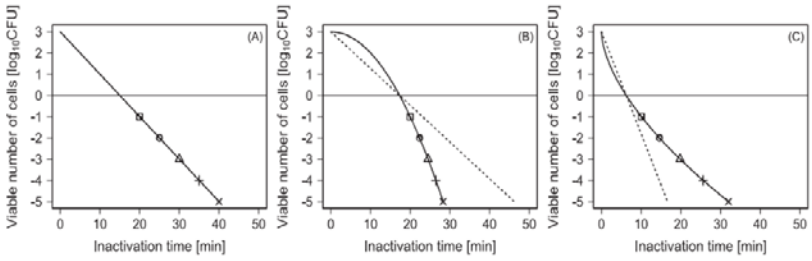

Fig. 7 – Inactivation kinetic models and probability of a population containing survivors calculation using inactivation kinetics (Koyama *et al.*, 2019).

According to a study by Kavuncuoglua *et al*., 2018, a mathematical model based on artificial neural networks (ANN) can be used to calculate the diameter of the inhibitory area of the nut extract for twelve different bacterial species.

The lowest activity was for *L. monocytogenes* for a concentration of 1/10 and the highest inhibition was observed for *A. hydrophila*.

For prediction of the inhibition zone (IZ) was chosen three independent variables (concentration (C), type of extraction (TE) and pathogens (P)). Table 1 indicated the statistical characteristics of the data and Table 2 demonstrated the correlation among the inhibition zones and independent variables.

Table 1
Selected parameters of statistical analysis

Variables	Mean	SD	Minimum	Maximum
Type of extraction	2.5	1.121	1	4
Concentration	0.462	0.343	0.1	1
Pathogens	6.5	3.461	1	12
Inhibition Zone (d_{inh} mm)	9.39	5.996	0	19.76

Table 2
Correlation coefficients between independent variables and dependent variable

	Type of extraction	Concentration	Pathogens
Inhibition zone (d_{inh} mm)	- 0.138	0.785	- 0.0671

The t- test of the statistical method shows the relationship between the predicted IZ values and the experimental IZ values for ANN in Table 3, Fig. 8 and Fig. 9.

Table 3
The statistical method t-test shows the relationship between the predicted IZ values and the experimental IZ values for ANN

Group name	N	Missing	Mean	Std dev	SEM	P value
Predicted IZ	29	0	2.045	0.563	0.105	0.669
Experimental output IZ	29	0	1.978	0.625	0.116	

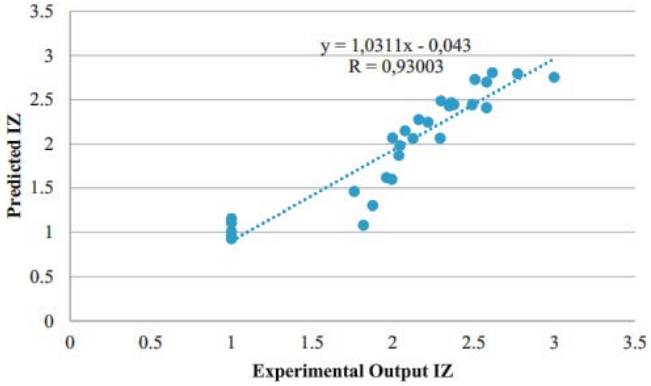

Fig. 8 – Scatter plot of experimental output versus predicted inhibition zone by ANN (Kavuncuoglua *et al.*, 2018).

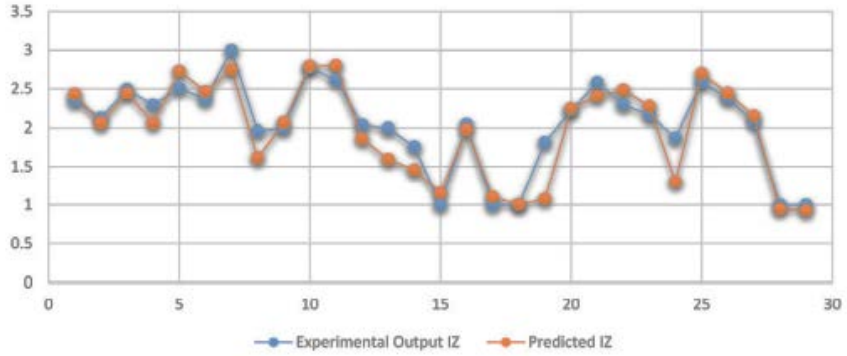

Fig. 9 – Predicted and actual output inhibition zone in Test Set for Levenberg–Marquardt algorithm for ANN (Kavuncuoglua *et al.*, 2018).

4. Conclusions

The properties of walnuts have been known since ancient times. All parts of the plant are important, such as: bark, leaves, dry skin and green skin of the fruit, septum and core.

Its properties are due to the presence of phytochemicals, such as flavonoids, carotenoids, alkaloids, nitrogen-containing compounds, polyphenols, Juglone, etc. In addition to the many benefits that walnut has on our health (antibacterial, antioxidant, antihistamine, analgesic, bronchodilator, antidiabetic, hepatoprotective, anti-inflammatory, antihypertensive, neuroprotective, wound healing), it also has important phytosanitary and insecticidal properties.

Also, the phytosanitary properties can be 20eyrick20 mathematically and the data can be interpreted through graphs.

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NUCUL (JUGLANS REGIA): UN REVIEW AL PROPRIETĂȚILOR FITOSANITARE ŞI MODELAREA LOR MATEMATICĂ

(Rezumat)

Nucul (*Juglans regia L.*) este cel mai răspândit nuc din lume. Toate părțile plantei sunt importante: coaja, frunzele, coaja uscată și verde a fructului, septul, miezul. Beneficiile nucilor se datorează prezenței unor substanțe fitochimice precum flavonoide, carotenoide, alcaloizi, polifenoli etc. Pe lângă numeroasele beneficii pe care nuca le are asupra sănătății noastre (antibacteriene, antioxidante), are și importante proprietăți

fitosanitare și insecticide. Nucile pot fi folosite datorită proprietăților lor prietenoase cu plantele sub formă de biopesticide care sunt sigure și pot fi o alternativă viabilă, ieftină și mai curată la produsele sintetice care pot fi dăunătoare mediului. Această lucrare de recenzie urmărește să aducă în prim-plan literatura disponibilă despre *Juglans regia* îndreptată asupra proprietăților antifungice și insecticide cu acțiune asupra plantelor și antibacteriene cu acțiune asupra organismului uman, precum și modelelor matematice privind multiplicarea microorganismelor.