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The Effect of Biotic Stress in Plant Species Induced by '*Candidatus* Phytoplasma solani'—An Artificial Neural Network Approach

Ivica Djalovic [1,*], Petar Mitrovic [1], Goran Trivan [2], Aleksandra Jelušić [2], Lato Pezo [3], Elizabet Janić Hajnal [4] and Tatjana Popović Milovanović [5]

[1] Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, 21101 Novi Sad, Serbia; petar.mitrovic@ifvcns.ns.ac.rs
[2] Institute for Multidisciplinary Research, University of Belgrade, 11030 Belgrade, Serbia; goran.trivan@imsi.bg.ac.rs (G.T.); jelusic.aleksandra@gmail.com (A.J.)
[3] Institute of General and Physical Chemistry, Studentski trg 12/V, 11000 Belgrade, Serbia; latopezo@yahoo.co.uk
[4] Institute of Food Technology, University of Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia; elizabet.janich@fins.uns.ac.rs
[5] Institute for Plant Protection and Environment, Teodora Drajzera 9, 11040 Belgrade, Serbia; tanjaizbis@gmail.com
* Correspondence: maizescience@yahoo.com

Abstract: Infections with phytoplasma present one of the most significant biotic stresses influencing plant health, growth, and production. The phytoplasma '*Candidatus* Phytoplasma solani' infects a variety of plant species. This pathogen impacts the physiological and morphological characteristics of plants causing stunting, yellowing, leaf curling, and other symptoms that can lead to significant economic losses. The aim of this study was to determine biochemical changes in peony (*Paeonia tenuifolia* L.), mint (*Mentha* × *piperita* L.), and dill (*Anethum graveolens* L.) induced by '*Ca*. Phytoplasma solani' in Serbia as well as to predict the impact of the biotic stress using artificial neural network (ANN) modeling. The phylogenetic position of the Serbian '*Ca*. Phytoplasma solani' strains originated from the tested hosts using 16S rRNA (peony and carrot strains) and *plsC* (mint and dill strains) sequences indicated by their genetic homogeneity despite the host of origin. Biochemical parameters significantly differed in asymptomatic and symptomatic plants, except for total anthocyanidins contents in dill and the capacity of peony and mint extracts to neutralize superoxide anions and hydroxyl radicals, respectively. Principal Component Analysis (PCA) showed a correlation between different chemical parameters and revealed a clear separation among the samples. Based on the ANN performance, the optimal number of hidden neurons for the calculation of TS, RG, PAL, LP, NBT, •OH, TP, TT, Tflav, Tpro, Tant, DPPH, and Car was nine (using MLP 8-9-13), as it produced high r^2 values (1.000 during the training period) and low SOS values. Developing an effective early warning system for the detection of plant diseases in different plant species is critical for improving crop yield and quality.

Keywords: '*Candidatus* Phytoplasma solani'; peony; mint; dill; carrot; biotic stress

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1. Introduction

Plants undergo a wide range of biotic and abiotic stresses that limit their proper growth [1]. Biotic stress in plants is caused by living organisms, including fungi, bacteria, phytoplasma, viruses, nematodes, insects, and weeds, causing diseases or damage [2]. Specific ion channels and kinase cascades and reactive oxygen species (ROS), as well as phytohormones (e.g., abscisic acid, salicylic acid, jasmonic acid, and ethylene), are activated in plants following exposure to stress [3]. Phytoplasmas are plant pathogenic bacteria belonging to the class Mollicutes. They lack cell walls and exclusively inhabit

the nutrient-rich phloem tissue of plants [4]. Diseases caused by phytoplasmas pose a threat to agricultural production by leading to substantial losses in yield and the quality of plants. More than 600 diverse diseases have been reported worldwide, mainly on vegetable crops belonging to the families *Apiaceae*, *Asteraceae*, *Cucurbitaceae*, *Fabaceae*, and *Solanaceae* [5–8]. The symptoms of diseases caused by phytoplasmas include yellowing or reddening of leaves, phyllody, stunting, virescence, shortened internodes, big bud, little leaf, witches' broom or proliferation, leaf curl, giant calyx, floral malformation, and vascular discoloration [7,9]. Phytoplasmas are transmitted from plant to plant by phloem-feeding insect vectors, and they propagate within the cytoplasm of both insects and plants. Phytoplasma attack causes various changes in terms of physiological, biochemical, and metabolic pathways [4,5,7,10,11]. For example, phytoplasma infections have been known to decrease chlorophyll synthesis [5,12] and protein content or to increase peroxidase activity [11]. Our previous studies [5,7] indicated biochemical changes in common evening-primrose (*Oenothera biennis* L.) and carrot (*Daucus carota* L.) resulting from infection by '*Candidatus* Phytoplasma solani'. Changes in *O. biennis* resulted in a significant increase in the peroxidation of lipids, phenylalanine ammonia-lyase activity, total sugars, polyphenols, and anthocyanins content, along with a decrease in photosynthetic pigments and total flavonoids [5]. The oxidative damage of membranes in carrot cells was accompanied by a decrease in the content of photosynthetic pigments, as well as a pronounced reduction in the level of glutathione (GSH) content. Conceivably, anthocyanidins were responsible for the enhanced antioxidative capacity [7].

Artificial neural networks (ANNs) have found applications in diverse domains for forecasting outcomes of intricate systems by processing input data. ANNs belong to a class of machine learning algorithms capable of learning from data and predicting results with precision and dependability, such as identifying plant diseases. ANNs have exhibited remarkable potential in predicting biotic stress in plants resulting from diverse pathogens, enabling real-time plant health monitoring and offering a pre-emptive alert mechanism for farmers, agronomists, and researchers to forestall disease outbreaks.

The present study aimed to determine biochemical changes induced by '*Ca*. Phytoplasma solani' in the three latest host plants identified in Serbia, including peony (*Paeonia tenuifolia* L.), mint (*Mentha* × *piperita* L.), and dill (*Anethum graveolens* L.), and to utilize these findings to construct an artificial neural network (ANN) model in order to predict the impact of the biotic stress in these particular plant species.

2. Materials and Methods

2.1. Plant Material

Experiments performed in this study were conceived for three plant species, i.e., *P. tenuifolia*, *Mentha* × *piperita*, and *A. graveolens* with externally visible disease symptoms caused by '*Ca*. Phytoplasma solani' (Figure 1) [13–15]. Plants of each species with symptoms (S–symptomatic) and without symptoms (A–asymptomatic) were collected from Bački Petrovac (45°21′38″ N 19°35′30″ E) in the Bačka region of Vojvodina, Serbia, at the "full-bloom" phenophase. The study field belongs to the Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia (Novi Sad, Serbia), for the purpose of reproduction and maintenance of seed collection.

A minimum of thirty plants of each tested species (per fifteen randomly selected asymptomatic and symptomatic plants) were collected at the growing sites. One part of the collected leaves (two per taken plant) was immersed in liquid nitrogen and further stored under −20 °C, while the other part was dried at ambient temperature in a well-ventilated place. Previously published results of biochemical changes in *D. carota* plants infected with '*Ca*. Phytoplasma solani' (Figure 1) in Serbia were used for the comparison in all assays [7].

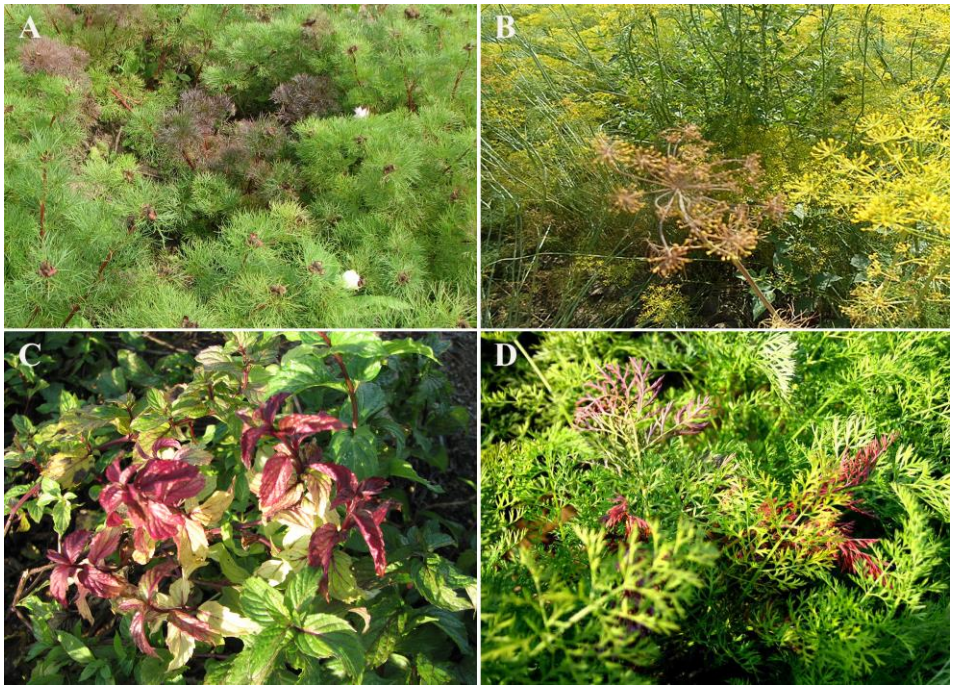

Figure 1. Symptoms caused by '*Ca.* Phytoplasma solani' on (**A**) peony—*P. tenuifolia*, (**B**) dill—*A. graveolens*, (**C**) mint—*Mentha* × *piperita*, and (**D**) carrot—*D. carota*.

2.2. Phylogenetic Position of the Tested Serbian 'Ca. Phytoplasma solani' Strains

Phylogenetic analysis was performed to check the position of the five Serbian '*Ca.* Phytoplasma solani' strains originating from the same *A. graveolens*, *P. tenuifolia*, *Mentha* × *piperita*, and *D. carota* plants as those used for biochemical analysis, in relation to *Phytoplasma* spp. strains from different hosts and countries. Two separate phylogenetic trees were constructed based on the previously published sequences of the Serbian strains of interest. The first Neighbor-Joining (NJ) phylogenetic tree was constructed based on the 16S rRNA sequences for the three Serbian '*Ca.* Phytoplasma solani' strains originating from *P. tenuifolia* (B15 and B18) [14] and *D. carota* (ML_NS-2016) [7], as well as with 23 comparative *Phytoplasma* spp. strains belonging to different 16Sr groups (Table 1) retrieved from the GenBank database. The tree was rooted with *Acholeplasma laidlawii* strain NCTC10116 (Table 1).

Table 1. List of *Phytoplasma* spp. strains from GenBank used to construct the phylogenetic tree based on 16S rRNA sequences.

Species	16Sr Group Classification	Strain	Isolation Source	Country	Acc. No.
'*Ca.* Phytoplasma solani'	XII-A	B15 [a]	*P. tenuifolia*	Serbia	KC960487
		B18 [a]	*P. tenuifolia*	Serbia	KF614623
		ML_NS-2016 [a]	*D. carota*	Serbia	MF503627
		Ei22	*Euscelis incisus*	Serbia	MN047263
		grape8	*Vitis vinifera*	Iran	MK392488
		Amaranthus26	*Amaranthus* sp.	-	MN007088
		NSRTCPso6	*Prunus domestica*	Jordan	MH085229
		Rus-AWB804F	*Medicago sativa*	Russia	KY587525
		Kz22	*Citrus* sp.	Iran	MG563790
		P8	*Solanum tuberosum*	Germany	PP261349
		284/09	*Nicotiana tabacum*	-	NC_022588
Strawberry lethal yellows phytoplasma (CPA)	XII-B variant	NZSb11	-	Australia and New Zealand	CP002548

Table 1. *Cont.*

Species	16Sr Group Classification	Strain	Isolation Source	Country	Acc. No.
Aster yellows witches'-broom phytoplasma	I-A	AYWB	*Lactuca sativa*	USA	CP000061
'*Catharanthus roseus*' aster yellows phytoplasma	I-B	De Villa	*Catharanthus roseus*	South Africa	CP035949
Maize bushy stunt phytoplasma	I-B	M3	*Dalbulus maidis*	Brazil	CP015149
Paulownia witches'-broom phytoplasma	I-D	Zhengzhou	*Paulownia*	China	CP066882
Peanut witches'-broom phytoplasma	II-A	T48	*Areca catechu*	China	OR239773
'*Echinacea purpurea*' witches'-broom phytoplasma	II-A	NCHU2014	*Catharanthus roseus*	Taiwan	CP040925
'*Parthenium* sp.' phyllody phytoplasma	II-D	PR08	*Parthenium hysterophorus*	India	CP097207
'*Ca.* Phytoplasma ziziphi'	V-B	Jwb-nky	*Ziziphus jujuba*	China	CP025121
'*Ca.* Phytoplasma luffae'	VIII-A	NCHU2019	*Luffa aegyptiaca*	Taiwan	CP054393
'*Ca.* Phytoplasma mali'	X-A	AT	-	-	CU469464
'*Ca.* Phytoplasma oryzae'	XI-A	HN2022	-	China	CP116038
Acholeplasma laidlawii [b]	-	NCTC10116	-	-	LS483439

[a] Serbian *Ca.* Phytoplasma solani strains of interest in this study; [b] outgroup.

The second NJ phylogenetic tree was constructed based on the plsC gene (1-acyl-sn-glycerol-3-phosphate acyltransferase) sequences with the remaining two Serbian '*Ca.* Phytoplasma solani' strains of interest, originating from *A. graveolens* (STOL2) [13] and *Mentha* × *piperita* (STOL3) [15], and 13 comparative *Phytoplasma* spp. strains from different hosts and countries (Table 2), which were also from GenBank. This tree was rooted with *Acholeplasma laidlawii* strain DSM 23060 (Table 2).

Table 2. List of *Phytoplasma* spp. strains from GenBank used to construct the phylogenetic tree based on *plsC* gene sequences.

Species	Strain	Isolation Source	Country	Acc. No.
'*Ca.* Phytoplasma solani'	STOL2 [a]	*A. graveolens*	Serbia	KT281866
	STOL3 [a]	*Mentha* × *piperita*	Serbia	KT281865
	284/09	*Nicotiana tabacum*	-	FO393427
	231/09	-	-	FO393428
	Stol11_3_C	*Convolvulus arvensis*	-	JQ977746
	Stol11_1_C	*Convolvulus arvensis*	-	JQ977744
	Stol11_2_U	*Urtica dioica*	-	JQ977745
'*Rubus fruticosus*' stolbur phytoplasma	Stol11-Rubus1/2010-Bg	*Rubus fruticosus*	Bulgaria	JN561701
'*Convolvulus arvensis*' stolbur phytoplasma	Stol11-Conv2/2010-Bg	*Convolvulus arvensis*	Bulgaria	JN561700
'*Convolvulus arvensis*' stolbur phytoplasma	Stol11-Conv12/2011-Bg	*Convolvulus arvensis*	Bulgaria	JN561699
Lavender decline stolbur phytoplasma	Stol 11	-	-	AF447596
Strawberry lethal yellows phytoplasma (CPA)	NZSb11	-	-	CP002548
'*Ca.* Phytoplasma australiense'	-	-	-	AM422018
Acholeplasma laidlawii [b]	DSM 23060			NZ_QRDS01000001

[a] Serbian '*Ca.* Phytoplasma solani' strains of interest in this study; [b] outgroup.

Before the construction of each phylogenetic tree, sequences of all strains were aligned to the same size (710 nt for 16S rRNA; 399 nt for plsC) in the BioEdit v.7.0 program using the ClustalW Multiple alignment function. Trees were constructed in Mega7 software, using the bootstrap value of 1000. The Kimura two-parameter nucleotide substitution model was used to compute genetic distances [14].

2.3. Biochemical Analyses of Peony, Mint, Dill, and Carrot Infected by 'Ca. Phytoplasma solani'

Biochemical analysis includes the determination of parameters related to total sugars (TS), reduced glutathione (GSH), lipid peroxidation intensity (LP), phenylalanine ammonia-lyase (PAL) activity, photosynthetic pigments (total chlorophyll a and b and carotenoids), total polyphenolics (TP) and polyphenolic groups (Tflav-flavonoids, TT-tannins, Tpro-proanthocyanidins, Tant-anthocyanidins), and antioxidant capacity. All methods stated were explained in great detail within their respective references, as well as in our previous papers [7,16]. Total sugars (TS), or precisely, total carbohydrate content expressed as mg glucose equivalents/g fresh weight (fw), were determined from aqueous extracts with sulfuric acid–UV method [15]. Reduced glutathione (GSH) [17] and lipid peroxidation intensity (LP) [18] were determined from trichloroacetic acid extracts (5 and 20%, respectively), while 0.05 M sodium borate buffer was used for phenylalanine ammonia-lyase (PAL) activity [19]. These parameters were expressed as µmol GSH/g fw, nmol MDA equivalents/g fw, and U/g fw for GSH, LP, and PAL, respectively. Photosynthetic pigments (total chlorophyll a and b and carotenoids) were extracted with 80% acetone, as determined by the Von Wettstein (1957) [20] method and expressed as mg/g dry weight (dw). Total polyphenolics (TP) and polyphenolic groups (Tflav-flavonoids, TT-tannins, Tpro-proanthocyanidins, Tant-anthocyanidins) and antioxidant tests were extracted with an acidified methanolic solution ($MeOH:H_2O:CH_3COOH$, 140:50:10). All total polyphenolic groups were expressed as mg/g dw and determined by methods explained in detail in following references: TP, TT, and TPro in Makkar (2003) [21] and TFlav in Pękal and Pyrzynska (2014) [22] and Tant by Lee et al. (2005) [23]. Antioxidant capacity was determined through three antioxidant tests, including NBT, $^{\bullet}OH$, and DPPH, by testing the scavenging of 1.1-diphenyl-2-picrylhydrazyl free radicals, superoxide anions ($O_2^{\bullet -}$), and hydroxyl radicals ($^{\bullet}OH$), respectively. Inhibition (%) of these reactive species in comparison to the control was determined according to Panda (2012) [24], Ahmed (2013) [25], and Sánchez-Moreno (2002) [26], respectively.

2.4. Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a statistical method commonly used in exploratory data analysis [27] to identify patterns and relationships within a dataset. The transformation is based on the eigenvalue decomposition of a data correlation matrix [28], which ensures that the first principal component captures the highest possible variance in the data. This technique can reveal spatial relationships among processing parameters and facilitate the identification of underlying patterns within complex datasets.

2.5. Artificial Neural Network (ANN) Modeling

During the training cycle for artificial neural network (ANN) modeling, all input and output data were normalized to improve the network's behavior. The optimal number of hidden layers and neurons in each layer was determined through trial and error to achieve good performance. A multi-layer perceptron (MLP) model with three layers (input, hidden, and output) was chosen for this study, as it is known to be effective at approximating nonlinear functions [29]. In this study, the optimal number of hidden neurons was found to be ten. The ANN scheme is presented in Figure 2. The Broyden–Fletcher–Goldfarb–Shanno (BFGS) algorithm was used for ANN modeling, and the training process was repeated multiple times to achieve the best performance considering parameter variability. Successful training was determined when the learning and cross-validation curves (Sum of Squares vs. training cycles) approached zero. The ANN was tested using the best weights obtained during the training step, and the coefficient of determination (r^2) and Sum of

Squares (SOS) were used to evaluate the performance (i.e., accuracy) of the ANN. Artificial neural networks show high performance in predicting TS, RG, PAL, LP, TP, TT, Tflav, Tpro, Tant, NBT, •OH, DPPH, Tcha, Tchb, and Car by calculating weight coefficients and biases with respect to characteristic input parameters (logical variables including plant type and treatment) [30,31]. The basic equation (Equation (1)) for calculating the output data of artificial neural networks is as follows:

$$Y = f_1(W_2 \cdot f_2(W_1 \cdot X + B_1) + B_2) \quad (1)$$

where Y represents the output value, f_1 and f_2 represent the transfer function in the hidden and output layer, and X represents the matrix of the input layer [30].

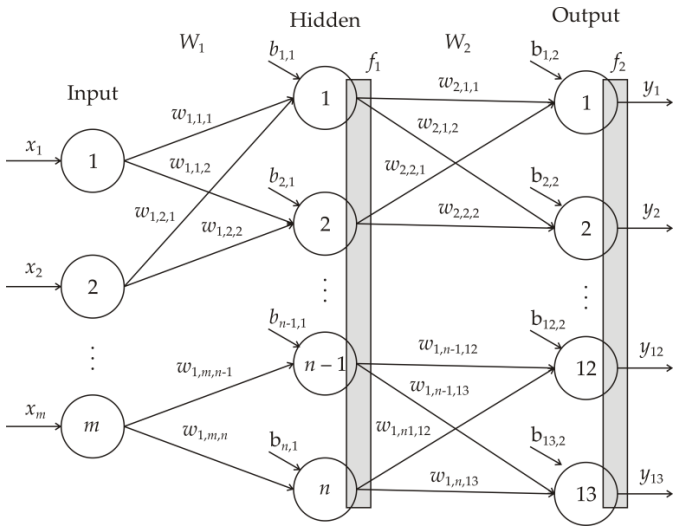

Figure 2. ANN topology with three layers (input, output, and hidden) with weights, biases, and transfer functions.

2.6. Global Sensitivity Analysis

Yoon's global sensitivity equation was used to calculate the relative impact of the input parameters on the output variables, according to the weight coefficients of the developed ANN models [32,33]:

$$RI_{ij}(\%) = \frac{\sum_{k=0}^{n} (w_{ik} \cdot w_{kj})}{\sum_{i=0}^{m} \left| \sum_{k=0}^{n} (w_{ik} \cdot w_{kj}) \right|} \cdot 100\% \quad (2)$$

where w—weight coefficient in the ANN model, i—input variable, j—output variable, k—hidden neuron, n—number of hidden neurons, and m—number of inputs.

3. Results

3.1. Phylogenetic Position of 'Ca. Phytoplasma solani' Strains

The NJ phylogenetic tree based on the 16S rRNA sequences of three tested Serbian '*Ca.* Phytoplasma solani' strains from *P. tenuifolia* (B15 and B18) and *D. carota* (ML_NS-2016) and the comparative *Phytoplasma* spp. strains from different hosts/countries is presented in Figure 3. Based on the constructed tree, all tested/comparative '*Ca.* Phytoplasma solani' strains were placed in the same tree cluster (the XII-A 16Sr Group) showing genetic homogeneity among themselves regardless of the host/country of origin. The remaining *Phytoplasma* spp. strains belonging to 16Sr Groups I (A, B, and D), II (A and D), VIII-A, X-A, XI-A, and XII-B were clearly separated in other tree branches/clusters, each corresponding to a specific 16Sr Group. *Acholeplasma laidlawii* strain NCTC10116 was placed on a monophyletic tree branch, as an outgroup (Figure 3).

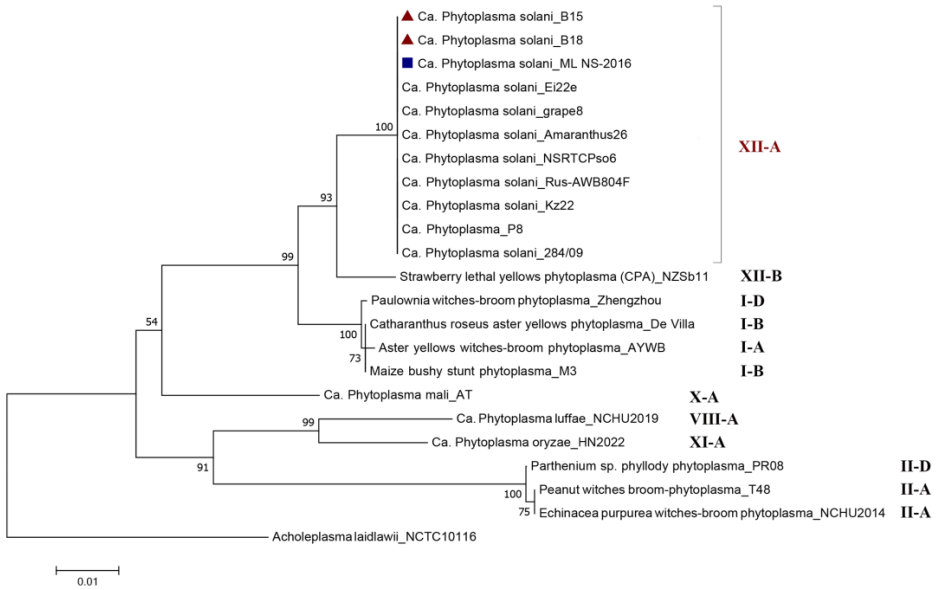

Figure 3. Neighbor-joining phylogenetic tree showing the position of the three tested Serbian '*Ca*. Phytoplasma solani' strains from *P. tenuifolia* ▲ and *D. carota* ■ and 23 comparative *Phytoplasma* spp. strains belonging to different 16Sr Groups. *Acholeplasma laidlawii* strain NCTC10116 from the GenBank database served as an outgroup.

The NJ phylogenetic tree that was constructed based on the *plsC* gene sequences for the two tested Serbian '*Ca*. Phytoplasma solani' strains from *A. graveolens* and *Mentha* × *piperita* and comparative *Phytoplasma* spp. strains from different hosts/countries is presented in Figure 4. According to the constructed tree, all Serbian tested '*Ca*. Phytoplasma solani' as well as comparative '*Ca*. Phytoplasma solani' strains and stolbur phytoplasmas were genetically homogenous and placed in the same cluster. Based on the same gene, sequences of Strawberry lethal yellows phytoplasma (CPA) strain NZSb11 and '*Ca*. Phytoplasma australiense' strains were identical; therefore, these two strains were grouped together within the second cluster of the tree. An outgroup *Acholeplasma laidlawii* strain DSM23060 was placed on a monophyletic tree branch.

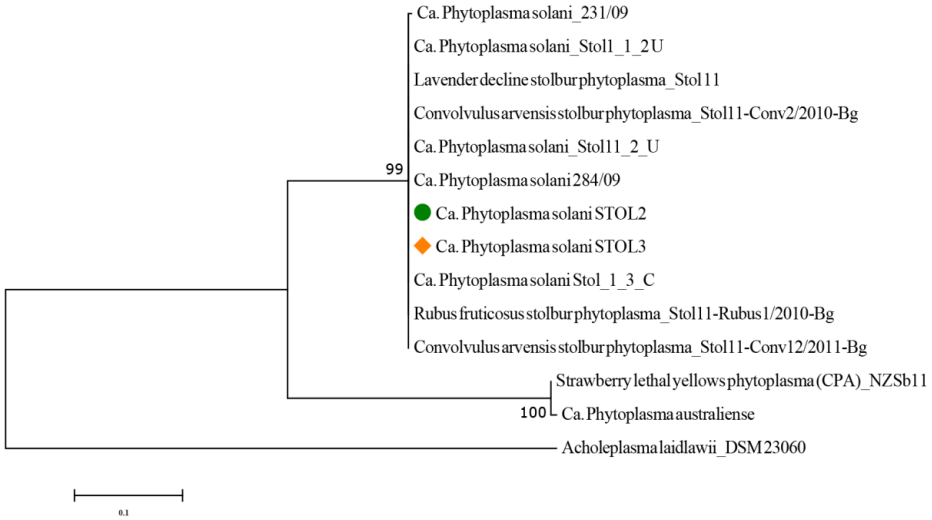

Figure 4. Neighbor-joining phylogenetic tree showing the position of the two tested Serbian '*Ca*. Phytoplasma solani' strains from *A. graveolens* ● and *Mentha* × *piperita* ◆ and 13 comparative *Phytoplasma* spp. strains according to sequences of the *plsC* gene. *Acholeplasma laidlawii* strain DSM23060 from the GenBank database served as an outgroup.

3.2. Biochemical Analyses of Peony, Mint, Dill, and Carrot Infected by 'Ca. Phytoplasma solani'

There was a significant difference for most of the tested biochemical parameters when asymptomatic and symptomatic plants were compared, except for total anthocyanidins contents in dill and the capacity of peony and mint extracts to neutralize superoxide anions and hydroxyl radicals, respectively (Table 3).

The total sugars content accumulated in symptomatic leaves was twice the amount of the sugars in healthy leaves. Lipid peroxidation intensity was clearly and dramatically higher in the leaves of symptomatic plants, reaching twice the values of LP for the asymptomatic dill and peony plants. Reduced glutathione content was enhanced slightly but significantly in all tested species. Although the activity of PAL was markedly higher in the symptomatic leaves (1.25–3.5 times higher), only in mint and dill were the amounts of phenolic compounds positively correlated with this trend (possibly because of higher tannin and proanthocyanidins contents); however, the amount of phenolics in peony plants was lower in symptomatic leaves (except for anthocyanidins content) than in those of the other two species. Photosynthetic pigment contents (chlorophyll a and b and carotenoids) significantly decreased (2.45–5.08 times) in the leaves of all tested species. Although there was a difference in antioxidant capacity between asymptomatic and symptomatic plants within species, the mint and dill plants had enhanced antioxidant activity against superoxide anions (NBT test higher than 80% of neutralized radicals), while peony plants had pronounced antioxidant activity against DPPH radicals (DPPH test higher than 90% of neutralized radicals).

3.3. Principal Component Analysis (PCA)

In this study, PCA was used to investigate the correlation between different chemical parameters tested, such as TS, RG, PAL, LP, TP, TT, Tflav, Tpro, Tant, NBT, $^{\bullet}$OH, DPPH, Tcha, Tchb, and Car. The plant samples, including mint AL, mint SL, dill AL, dill SL, peony AL, and peony SL, were observed, and the content of these variables was utilized in the PCA. The first three principal components generated by the PCA were utilized to display the results visually in a PCA graph, which effectively differentiated the six plant samples. By applying PCA to the provided dataset, it was possible to differentiate between the samples based on the processing parameters used. This technique was utilized as a tool in exploratory data analysis to characterize and distinguish the input parameters for the neural network (as shown in Figure 5).

The results of the PCA (shown in Figure 5) reveal a clear separation between the samples, indicating good discrimination. The first three principal components (having eigenvalues of 5.15, 3.36, and 2.17), which account for 71.22% (the total variance explained by principal components was 34.34%; 22.42%, and 14.46%) of the total variability, were found to be sufficient for representing the data. The contents of TS (11.74%, based on correlations), PAL (9.80%), LP (8.68%), TP (11.60%), TT (11.53%), and DPPH (14.55%) had the most negative contributions to the calculation of the first factor coordinate (PC1), while the content of Tcha (7.01%) exerted the most positive influence on the PC1 coordinate calculation. Meanwhile, the content of RG (8.11%, based on correlations), Tflav (12.36%), Tcha (12.86%), and Tchb (12.80%) had negative influences on the second principal component (PCA), while Tpro (14.03%) and Car (11.38%) had positive influences on PC2. The content of LP (7.61%, based on correlations) and Tant (25.21%) had the most negative influence on the third principal component (PC3), while the content of NBT (9.03%), $^{\bullet}$OH (8.34%), TP (8.25%), TT (8.32%), and Tpro (9.26%) exerted the most positive influence on the calculation of the PC3 coordinate.

According to the PCA, the carrot SR and carrot AR samples were characterized by the highest Car content, while peony SL and peony AL were noted to obtain the most prominent value of DPPH, TS, TT TP, PAL, and RG content. The sample mint AL was characterized by augmented Tflav, Tcha, and Tchb contents.

Table 3. Biochemical parameters of healthy (A—asymptomatic) and '*Ca*. Phytoplasma solani'-infected (S—symptomatic) leaves of mint (*Mentha* × *piperita*), dill (*A. graveolens*), and peony (*P. tenuifolia*) plants. Data on carrot (*D. carota*) plants served as a comparison in this study.

		Carrot [a] (*Daucus carota*)		**Mint (*Mentha* × *piperita*)**		**Dill (*Anethum graveolens*)**		**Peony (*Paeonia tenuifolia*)**	
		A	**S**	**A**	**S**	**A**	**S**	**A**	**S**
Total sugars (% fw)	$\overline{X} \pm Se$	5.79 ± 0.01	5.15 ± 0.01	3.45 ± 0.08	6.62 ± 0.01	3.44 ± 0.02	5.15 ± 0.01	4.80 ± 0.01	11.98 ± 0.01
	t-test A/S	*		*		*		*	
Reduced glutathione (μmol GSH/g fw)	$\overline{X} \pm Se$	2.75 ± 0.01	3.10 ± 0.01	3.28 ± 0.01	3.93 ± 0.01	4.21 ± 0.01	5.07 ± 0.02	4.55 ± 0.01	5.01 ± 0.01
	t-test A/S	*		*		*		*	
Phenylalanine ammonia-lyase (U/g fw)	$\overline{X} \pm Se$	258.82 ± 0.29	70.94 ± 0.03	164.33 ± 0.09	300.91 ± 0.06	247.63 ± 0.19	310.20 ± 0.42	413.51 ± 0.28	1450.21 ± 0.41
	t-test A/S	nd		*		*		*	
Lipid peroxidation (nmol MDA/g fw)	$\overline{X} \pm Se$	871.57 ± 0.26	1064.43 ± 3.80	590.77 ± 1.04	641.43 ± 0.30	597.37 ± 0.86	1476.27 ± 0.64	722.83 ± 0.12	1636.60 ± 1.70
	A/S	*		*		*		*	
Total polyphenols (mg/g dw)	$\overline{X} \pm Se$	4.03 ± 0.02	1.42 ± 0.01	4.10 ± 0.01	7.45 ± 0.06	5.17 ± 0.02	6.27 ± 0.01	94.02 ± 0.16	86.56 ± 0.23
	t-test A/S	*		*		*		*	
Total tannins (mg/g dw)	$\overline{X} \pm Se$	3.33 ± 0.04	1.18 ± 0.03	2.66 ± 0.02	5.83 ± 0.03	1.94 ± 0.01	2.34 ± 0.01	82.18 ± 0.02	77.35 ± 0.08
	t-test A/S	*		*		*		*	
Total flavonoids (mg/g dw)	$\overline{X} \pm Se$	0.043 ± 0.0	0.000 ± 0.0	0.263 ± 0.002	0.275 ± 0.002	0.032 ± 0.001	0.022 ± 0.001	0.050 ± 0.000	0.051 ± 0.001
	t-test A/S	nd		*		*		*	
Total proanthocyanidins (mg/g dw)	$\overline{X} \pm Se$	0.40 ± 0.0	6.02 ± 0.01	0.87 ± 0.01	0.79 ± 0.01	0.86 ± 0.01	2.20 ± 0.01	4.75 ± 0.01	3.61 ± 0.01
	t-test A/S	*		*		*		*	
Total anthocyanidins (mg/g dw)	$\overline{X} \pm Se$	0.003 ± 0.0	0.000 ± 0.0	0.003 ± 0.00	0.000 ± 0.00	0.009 ± 0.01	0.012 ± 0.00	0.003 ± 0.00	0.137 ± 0.00
	t-test A/S	nd		*		nd		*	
Total chlorophyll a (mg/g dw)	$\overline{X} \pm Se$	0.78 ± 0.01	0.54 ± 0.01	1.86 ± 0.01	0.46 ± 0.00	0.93 ± 0.01	0.27 ± 0.00	0.61 ± 0.01	0.12 ± 0.01
	t-test A/S	*		*		*		*	
Total chlorophyll b (mg/g dw)	$\overline{X} \pm Se$	0.18 ± 0.0	0.15 ± 0.0	0.77 ± 0.00	0.21 ± 0.01	0.42 ± 0.01	0.14 ± 0.00	0.24 ± 0.00	0.05 ± 0.00
	t-test A/S	*		*		*		*	
Carotenoids (mg/g dw)	$\overline{X} \pm Se$	0.36 ± 0.01	0.17 ± 0.0	0.39 ± 0.00	0.12 ± 0.00	0.35 ± 0.01	0.08 ± 0.00	0.29 ± 0.00	0.12 ± 0.00
	t-test A/S	*		*		*		*	

Table 3. *Cont.*

		Carrot [a] (*Daucus carota*)		Mint (*Mentha* × *piperita*)		Dill (*Anethum graveolens*)		Peony (*Paeonia tenuifolia*)	
		A	**S**	**A**	**S**	**A**	**S**	**A**	**S**
NBT test (% neutralized radicals)	$\overline{X} \pm Se$	20.33 ± 0.28	82.57 ± 0.23	93.53 ± 0.29	88.37 ± 0.09	82.10 ± 0.06	88.40 ± 0.06	44.30 ± 0.35	52.60 ± 0.23
	t-test A/S	*		*		*		nd	
•OH test (% neutralized radicals)	$\overline{X} \pm Se$	10.81 ± 2.42	0.69 ± 0.04	16.40 ± 0.15	15.17 ± 1.94	35.37 ± 1.28	4.70 ± 0.12	36.30 ± 0.46	30.90 ± 0.06
	t-test A/S	*		nd		*		*	
DPPH test (% neutralized radicals)	$\overline{X} \pm Se$	19.95 ± 0.02	10.90 ± 0.01	14.30 ± 0.01	15.43 ± 0.12	13.74 ± 0.08	22.80 ± 0.20	92.91 ± 0.06	90.87 ± 0.02
	t-test A/S	*		*		*		*	

*—difference between A and S according to the *t*-test ($p < 0.05$); nd—no difference. [a] carrot data retrieved from a previous study by Mitrović et al. [7] and not the primary result of this work.

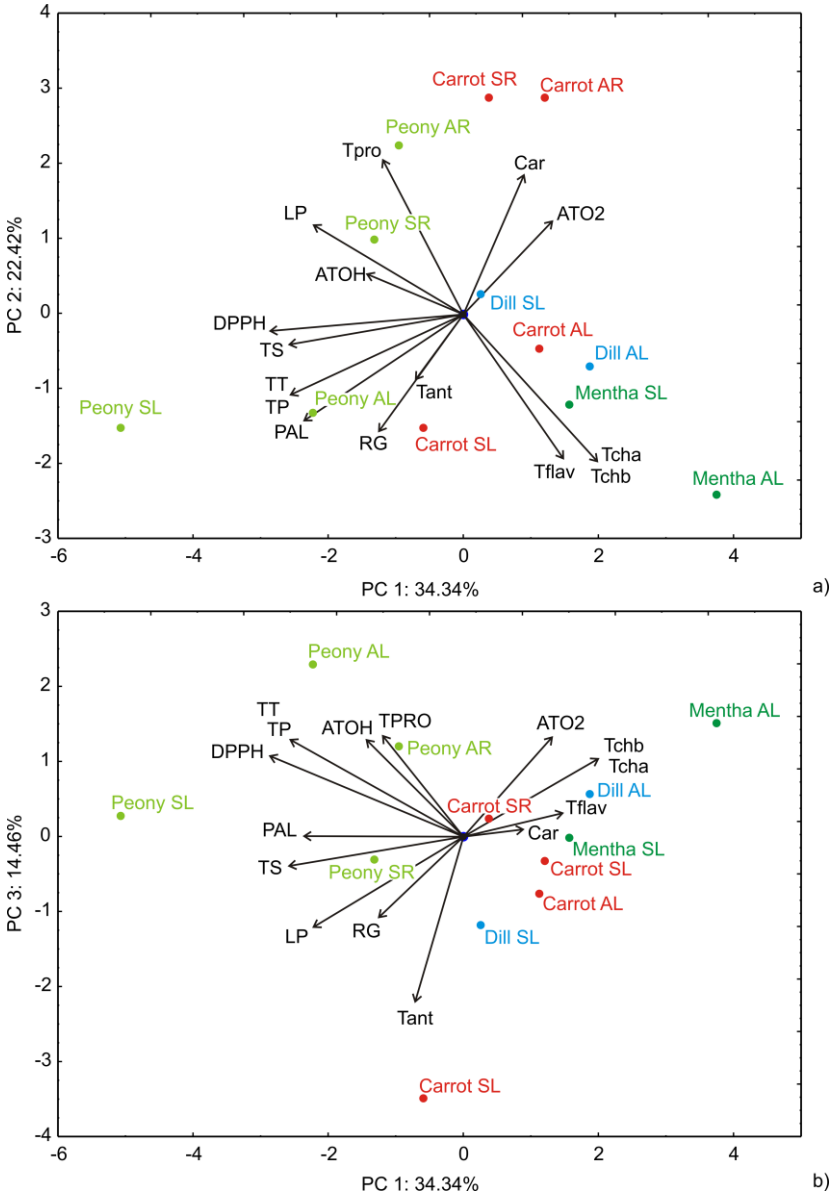

Figure 5. Biplot graph of chemical analysis for asymptomatic (A) and symptomatic (S) samples of the leaves of peony, mint, dill, and carrot.

3.4. Neurons in the ANN Hidden Layer

One of the crucial steps in designing an ANN is to determine the appropriate number of hidden layers and neurons in each layer, which depend on the complexity of the input–output relationship. As this relationship becomes more complex, more neurons need to be added to the hidden layer(s) [32]. The optimal number of hidden neurons was selected by minimizing the difference between the predicted and desired output values, using the Sum of Squares (SOS) as the performance indicator during testing. The used MLP model was identified according to StatSoft Statistica's notation, which indicates the number of inputs, neurons in the hidden layer(s), and outputs. Based on the ANN performance, it was observed that the optimal number of hidden neurons for the calculation of TS, RG, PAL, LP, NBT, •OH, TP, TT, Tflav, Tpro, Tant, DPPH, and Car was nine (using MLP 8-9-13), as it produced high r^2 values (1.000 during the training period) and low SOS values. The optimal networks used for predicting TS, RG, PAL, LP, NBT, •OH, TP, TT, Tflav, Tpro, Tant, DPPH, and Car were able to produce reasonably accurate output values across a wide range of process variables. In most cases, the predicted values were very close to the experimental

or target values, as evidenced by the high r^2 values obtained from the ANN models. The complexity of the ANN models was quite high, with 211 weight biases required for TS, RG, PAL, LP, NBT, •OH, TP, TT, Tflav, Tpro, Tant, DPPH, and Car calculations. However, the models were able to fit the experimental data quite well, owing to the high nonlinearity of the system under investigation [31,32]. During the training period, the r^2 values between the experimental measurements and the ANN model outputs for TS, RG, PAL, LP, NBT, •OH, TP, TT, Tflav, Tpro, Tant, DPPH, and Car were equal to 1.000.

3.5. Sensitivity Analysis

A sensitivity analysis was conducted to determine the relative influence (RI) of inputs (such as plant type and treatment) on TS, RG, PAL, LP, NBT, •OH, TP, TT, Tflav, Tpro, Tant, DPPH, Tcha, Tchb, and Car, The results, shown in Figure 6, indicate how outputs change with respect to infinitesimal changes in inputs, reflecting both experimental errors and the inputs' influence on the outputs.

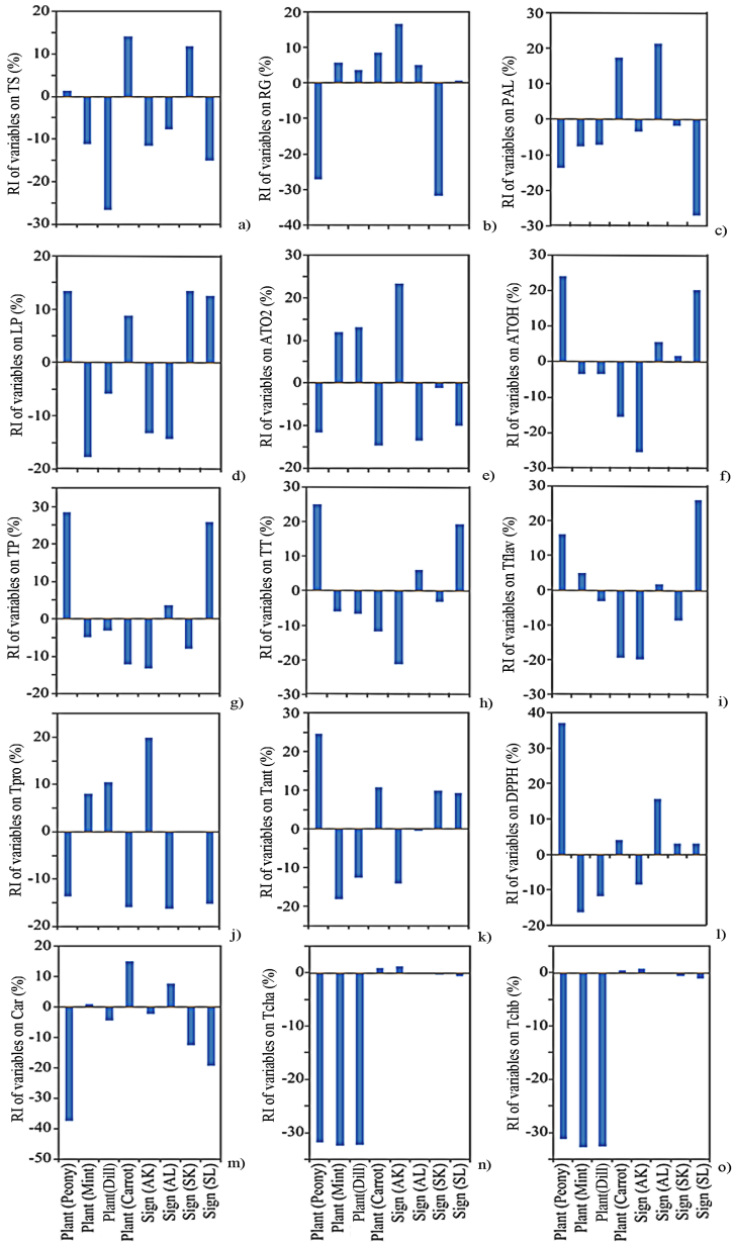

Figure 6. The relative influence of plant type and treatment on (**a**) TS, (**b**) RG, (**c**) PAL, (**d**) LP, (**e**) ATO2, (**f**) ATOH, (**g**) TP, (**h**) TT, (**i**) Tflav, (**j**) Tpro, (**k**) Tant, (**l**) DPPH, (**m**) Car, (**n**) Tcha, and (**o**) Tchb.

4. Discussion

Phytoplasmas belonging to the "stolbur" group 16SrXII-A ('*Ca*. Phytoplasma solani') are known to affect a wide range of wild and cultivated plants worldwide, causing severe economic damage [34]. Since its first report on pepper in Serbia in 1949, '*Ca*. Phytoplasma solani' has been described on diverse host plants (carrot, corn, grapevine, peony, peppermint, potato, etc.) in this country [12,35–40]. Species designation within phytoplasmas was primarily based on conserved 16S rRNA gene; however, since it does not allow distinctions among closely related *Ca*. Phytoplasma species, recent studies are encouraging the additional use of less conserved, housekeeping genes to enhance the resolving power [41]. However, the use of 16S rRNA sequences in this study allowed us to determine phylogenetic relatedness among the tested Serbian '*Ca*. Phytoplasma solani' strains. The performed phylogenetic analysis with 16S rRNA sequences of the three Serbian '*Ca*. Phytoplasma solani' strains from *P. tenuifolia* (B15 and B18) and *D. carota* (ML_NS-2016) and various reference *Phytoplasma* spp. strains belonging to different 16Sr Groups indicated an affiliation between the tested strains and the 16SrXII-A Group and genetic homogeneity with the other (tested/reference) '*Ca*. Phytoplasma solani' strains placed within this group, regardless of the host or country of origin. Additionally, earlier, *D. carota* strain ML_NS-2016 was confirmed to be genetically homogenous with different '*Ca*. Phytoplasma solani' strains originating from various countries (Bulgaria, Canada, China, France, Greece, Italy, Poland, Russia, Serbia, and Turkey) and hosts (corn, grapevine, parsley, parsnip, pea, periwinkle, potato, red sage, tobacco, tomato, and valerian), all belonging to the "stolbur" group (16SrXII-A) [7]. Similar to phylogenetic analysis based on 16S rRNA, a phylogenetic tree constructed based on the sequences of *plsC* gene, encoding the 1-acyl-sn-glycerol-3-phosphate acyltransferase enzyme involved in glycerophospholipid metabolism [42], placed the tested Serbian (*A. graveolens* strain STOL2 and *Mentha* × *piperita* strain STOL3) and comparative '*Ca*. Phytoplasma solani' strains in the same "stolbur" tree cluster. This gene was discriminatory enough to distinguish '*Ca*. Phytoplasma australiense' (first added as Strawberry lethal yellows phytoplasma) from the "stolbur" group. Some future research on Serbian '*Ca*. phytoplasma solani' strains should be directed toward the sequencing and analysis of multiple housekeeping genes such as *tuf*, *secY*, *vmp1*, *stamp*, etc. [8,43], which could eventually reveal some genetic differences.

Artificial neural networks (ANNs) have found application in diverse domains for forecasting the outcomes of intricate systems by processing input data. ANNs belong to a class of machine learning algorithms capable of learning from data and predicting results with precision and dependability, such as identifying plant diseases. ANNs have exhibited remarkable potential in predicting biotic stress in plants resulting from diverse pathogens, enabling real-time plant health monitoring and offering a pre-emptive alert mechanism for farmers, agronomists, and researchers to forestall disease outbreaks. Developing an effective early warning system for the detection of plant diseases in different plant species is critical for improving crop yield and quality. The identification and prevention of diseases of crops are essential for improving crop prediction. Based on our results, the ANN model can be considered as an efficient computational methodology for modeling and predicting the effect of the biotic stress in different plants induced by '*Ca*. Phytoplasma solani'.

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References

1. Ghosh, S.; Dey, G. Biotic and abiotic stress tolerance through CRISPR-Cas mediated genome editing. *J. Plant Biochem. Biotechnol.* **2022**, *31*, 227–238. [CrossRef]
2. Prasad, A.; Senthil-Kumar, M.; Prasad, M. Complex molecular mechanisms determine fitness of plants to biotic and abiotic stresses. *J. Plant Biochem. Biotechnol.* **2021**, *30*, 633–635. [CrossRef]
3. Ben Rejeb, I.; Pastor, V.; Mauch-Mani, B. Plant responses to simultaneous biotic and abiotic stress: Molecular mechanisms. *Plants* **2014**, *3*, 458–475. [CrossRef] [PubMed]
4. Ayvacı, H.; Güldür, M.E.; Dikilitas, M. Physiological and Biochemical Changes in Lucerne (*Medicago sativa*) Plants Infected with 'Candidatus *Phytoplasma australasia*'-Related Strain (16SrII-D Subgroup). *Plant Pathol. J.* **2022**, *38*, 146–158. [CrossRef] [PubMed]
5. Kiprovski, B.; Đalović, I.; Adamović, D.; Mitrović, P.; Marjanović-Jeromela, A.; Malenčić, Đ.; Popović, T. Biochemical changes in *Oenothera biennis* plants infected by '*Candidatus* Phytoplasma solani'. *J. Plant Pathol.* **2018**, *100*, 209–214. [CrossRef]
6. Kumari, S.; Nagendran, K.; Rai, A.B.; Singh, B.; Rao, G.P.; Bertaccini, A. Global status of phytoplasma diseases in vegetable crops. *Front. Microbiol.* **2019**, *10*, 1349. [CrossRef] [PubMed]
7. Mitrovic, P.; Djalovic, I.; Kiprovski, B.; Veljović Jovanović, S.; Trkulja, V.; Jelušić, A.; Popović, T. Oxidative stress and antioxidative activity in leaves and roots of carrot plants induced by *Candidatus* Phytoplasma solani. *Plants* **2021**, *10*, 337. [CrossRef]
8. Popović, T.; Mitrović, P.; Kosovac, A. Molecular characterization of *Candidatus* Phytoplasma solani in celery: Case study in Futog. *Field Veg. Crop. Res.* **2021**, *58*, 66–71. [CrossRef]
9. Mitrović, M.; Marinković, S.; Cvrković, T.; Jović, J.; Krstić, O.; Jakovljević, M. Framework for risk assessment of 'Candidatus Phytoplasma solani'associated diseases outbreaks in agroecosystems in Serbia. *J. Plant Pathol.* **2022**, *104*, 537–552. [CrossRef]
10. Zafari, S.; Niknam, V.; Musetti, R.; Noorbakhsh, S.N. Effect of phytoplasma infection on metabolite content and antioxidant enzyme activity in lime (*Citrus aurantifolia*). *Acta Physiol. Plant.* **2012**, *34*, 561–568. [CrossRef]
11. Hameed, S.; Akhtar, K.P.; Hameed, A.; Gulzar, T.; Kiran, S.; Yousaf, S.; Abbas, G.; Asghar, M.J.; Sarwar, N. Biochemical changes in the leaves of mungbean (*Vigna radiata*) plants infected by phytoplasma. *Turk. J. Biochem.* **2017**, *42*, 591–599. [CrossRef]
12. Junqueira, A.; Bedendo, I.; Pascholati, S. Biochemical changes in corn plants infected by the maize bushy stunt phytoplasma. *Physiol. Mol. Plant Pathol.* **2004**, *65*, 181–185. [CrossRef]
13. Trkulja, V.; Adamović, D.; Đalović, I.; Mitrović, P.; Kovačić-Jošić, D.; Lukač, Z.; Komić, J. First report of stolbur phytoplasma associated with *Anethum graveolens* in Serbia. *Plant Dis.* **2016**, *100*, 516. [CrossRef]
14. Adamovic, D.; Djalovic, I.; Mitrovic, P.; Kojic, S.; Pivic, R.; Josic, D. First Report on Natural Infection of *Paeonia tenuifolia* by '*Candidatus* Phytoplasma solani'in Serbia. *Plant Dis.* **2014**, *98*, 565. [CrossRef]
15. Mitrović, P.; Trkulja, V.; Adamović, D.; Đalović, I.; Milovac, Ž.; Kovačić-Jošić, D.; Mihić Salapura, J. First report of stolbur phytoplasma on Mentha× piperita in Serbia. *Plant Dis.* **2016**, *100*, 853. [CrossRef]
16. Kimura, M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **1980**, *16*, 111–120. [CrossRef]
17. Albalasmeh, A.A.; Berhe, A.A.; Ghezzehei, T.A. A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. *Carbohydr. Polym.* **2013**, *97*, 253–261. [CrossRef]
18. Rahman, I.; Kode, A.; Biswas, S.K. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. *Nat. Protoc.* **2006**, *1*, 3159–3165. [CrossRef]
19. Hodges, D.M.; DeLong, J.M.; Forney, C.F.; Prange, R.K. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta* **1999**, *207*, 604–611. [CrossRef]
20. Gerasimova, N.G.; Pridvorova, S.M.; Ozeretskovskaya, O.L. Role of L–phenylalanine ammonia Lyase in the induced resistance and susceptibility of sotato plants. *Appl. Biochem. Microbiol.* **2005**, *41*, 103–105. [CrossRef]
21. Von Wettstein, D. Chlorophyll-letale und der submikroskopische Formwechsel der plastiden. *Exp. Cell Res.* **1957**, *12*, 427–506. [CrossRef] [PubMed]
22. Makkar, H.P.S. *Quantification of Tannins in Tree and Shrub Foliage: A Laboratory Manual*; Springer Science & Business Media: Berlin/Heidelberg, Germany, 2003.
23. Pękal, A.; Pyrzynska, K. Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Anal. Methods* **2014**, *7*, 1776–1782. [CrossRef]

24. Lee, J.; Durst, R.W.; Wrolstad, R.E. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants and wines by the pH differential method: Collaborative study. *J. AOAC Int.* **2005**, *88*, 1269–1278. [CrossRef] [PubMed]
25. Panda, S.K. Assay guided comparison for enzymatic and nonenzymatic antioxidant activities with special reference to medicinal plants. In *Antioxidant Enzyme*, 3rd. ed.; El-Missiry, M.A., Ed.; IntechOpen: Rijeka, Croatia, 2012; Volume 14, pp. 382–400.
26. Ahmed, D.; Saman, Z.; Hira, B. In vitro analysis of antioxidant activities of *Oxalis corniculata* Linn. fractions in various solvents. *Afr. J. Tradit. Complement. Altern. Med.* **2013**, *10*, 158–165. [CrossRef]
27. Sánchez-Moreno, C. Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Int. J. Food Sci. Technol.* **2002**, *8*, 121–137. [CrossRef]
28. Brlek, T.; Pezo, L.; Voća, N.; Krička, T.; Vukmirović, Đ.; Čolović, R.; Bodroža-Solarov, M. Chemometric approach for assessing the quality of olive cake pellets. *Fuel Process. Technol.* **2013**, *116*, 250–256. [CrossRef]
29. Abdi, H.; Williams, L.J. Principal component analysis. *Wiley Interdiscip. Rev. Comput. Stat.* **2010**, *2*, 433–459. [CrossRef]
30. Hu, X.; Weng, Q. Estimating impervious surfaces from medium spatial resolution imagery using the self-organizing map and multi-layer perceptron neural networks. *Remote Sens. Environ.* **2009**, *113*, 2089–2102. [CrossRef]
31. Karlović, S.; Bosiljkov, T.; Brnčić, M.; Jezek, D.; Tripalo, B.; Dujmić, F.; Dzineva, I.; Skupnjak, A. Comparison of artificial neural network and mathematical models for drying of apple slices pretreated with high intensity ultrasound. *Bulg. J. Agric. Sci.* **2013**, *19*, 1372–1377.
32. Chattopadhyaya, P.B.; Rangarajana, R. Application of ANN in sketching spatial nonli-nearity of unconfined aquifer in agricultural basin. *Agric. Water Manag.* **2014**, *133*, 81–91. [CrossRef]
33. Yoon, Y.; Swales, G., Jr.; Margavio, T.M. A Comparison of Discriminant Analysis versus Artificial Neural Networks. *J. Oper. Res. Soc.* **1993**, *44*, 51–60. [CrossRef]
34. Quaglino, F.; Zhao, Y.; Casati, P.; Bulgari, D.; Bianco, P.A.; Wei, W.; Davis, R.E. '*Candidatus* Phytoplasma solani', a novel taxon associated with stolbur-and bois noir-related diseases of plants. *Int. J. Syst. Evol. Microbiol.* **2013**, *63*, 2879–2894. [CrossRef] [PubMed]
35. Mitrović, M.; Jakovljević, M.; Jović, J.; Krstić, O.; Kosovac, A.; Trivellone, V.; Jermini, M.; Toševski, I.; Cvrković, T. 'Candidatus phytoplasma solani'genotypes associated with potato stolbur in Serbia and the role of *Hyalesthes obsoletus* and *Reptalus panzeri* (hemiptera, cixiidae) as natural vectors. *Eur. J. Plant Pathol.* **2016**, *144*, 619–630. [CrossRef]
36. Martinović, M.; Bjegović, P. O nekim bolestima i štetočinama utvrđenim u NR Srbiji u 1949 godini. *Zaštita Bilja* **1950**, *2*, 59–68. (In Serbian)
37. Duduk, B.; Botti, S.; Ivanović, M.; Krstić, B.; Dukić, N.; Bertaccini, A. Identification of phytoplasmas associated with grapevine yellows in Serbia. *J. Phytopathol.* **2004**, *152*, 575–579. [CrossRef]
38. Duduk, B.; Bertaccini, A. Corn with symptoms of reddening: New host of stolbur phytoplasma. *Plant Dis.* **2006**, *90*, 1313–1319. [CrossRef] [PubMed]
39. Jović, J.; Ember, I.; Mitrović, M.; Cvrković, T.; Krstić, O.; Krnjajić, S.; Acs, Z.; Kolber, M.; Toševski, I. Molecular detection of potato stolbur phyto-plasma in Serbia. *Bull. Insectology* **2011**, *64*, S83–S84.
40. Mitrović, J.; Pavlović, S.; Duduk, B. Survey and Multigene Characterization of Stolbur Phytoplasmas on Various Plant Species in Serbia. *Phytopathol. Mediterr.* **2013**, *52*, 8. [CrossRef]
41. Martini, M.; Quaglino, F.; Bertaccini, A. Multilocus Genetic Characterization of Phytoplasmas. In *Phytoplasmas: Plant Pathogenic Bacteria-III: Genomics, Host Pathogen Interactions and Diagnosis*; Springer: Singapore, 2019; pp. 161–200.
42. Böhm, J.W.; Duckeck, D.; Duduk, B.; Schneider, B.; Kube, M. Genome Comparison of 'Candidatus Phytoplasma rubi'with Genomes of Other 16SrV Phytoplasmas Highlights Special Group Features. *Appl. Microbiol.* **2023**, *3*, 1083–1100. [CrossRef]
43. Contaldo, N.; Stepanović, J.; Pacini, F.; Bertaccini, A.; Duduk, B. Molecular Variability and Host Distribution of 'Candidatus Phytoplasma Solani' Strains from Different Geographic Origins. *Microorganisms* **2021**, *9*, 2530. [CrossRef]