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Antibacterial activity and QSAR modeling of natural monoterpenes against plant pathogens

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ABSTRACT

The antibacterial and the structure-activity relationship of ten natural monoterpenes against four plant pathogenic bacteria Erwinia carotovora, Ralstonia solanacearum, Rhodococcus fascians, and Rhizobium radiobacter was investigated. The antibacterial activity was evaluated in vitro by broth microdilution and agar dilution techniques as a minimum inhibitory concentration (MIC). The quantitative structure-activity relationships (QSARs) with some physicochemical descriptors of the tested monoterpenes were performed in order to investigate and predict the antibacterial activity. The results showed that the MICs depended on the bioassay test and the bacterial reduction rate increased with the increase of the concentration. Geraniol and thymol showed the highest potent activity among the tested monoterpenes against four plant pathogenic bacteria. The QSAR models showed excellent agreement between estimated and experimentally measured toxicity parameter (MIC) for the tested monoterpenes. The results of the current study could be used to identify or predict the best model for describing the antibacterial activity of new monoterpenes, the main constituents of plant essential oils, in the search for new biologically active agents against plant pathogenic bacteria.

Keywords: Monoterpenes; Antibacterial activity; Plant pathogens; MIC; QSAR.

INTRODUCTION

Plant pathogens bacteria are an important group of microorganisms that cause serious economically diseases of plants and their products throughout the world [1-3]. They cause plant diseases by extracellular digestion of plant tissues, ranging from spots, mosaics or pustules on leaves and fruits, or tuber rots to plant death. Some bacteria cause crown gall on leaves and shoots, a proliferation of plant cells that cause inflammation at the junction of the stem, soil and roots. Bacterial diseases are much more prevalent in sub-tropical and tropical regions of the world. The top 10 bacteria attack plants include, in rank order: *Pseudomonas syringae*, *Ralstonia solanacearum*, *Rhizobium radiobacter*, *Xanthomonas oryzae*, *X. campestris*, *X. axonopodis*, *Erwinia amylovora*, *E. carotovora*, *Xylella fastidiosa*, and *Dickeya* (*dadantii* and *solani*) [4].

Chemical bactericides provide the principal resources for controlling bacterial and fungal diseases of plants in pre- and postharvest phases. However, incessant use of destructive synthetic compounds has faced two major obstacles rising public concern regarding the contamination of consumable commodities with pesticide residues, and the increase of resistance in pathogen populations [5]. Therefore, there is a need for antimicrobial products that cause no harm to the environment and are non-toxic to mammals [6, 7].

Monoterpenes are the main constituents of plant essential oils and give plants their unique odoriferous properties as their low boiling points. These compounds are secondary metabolites that seem to play no major role in the metabolic functioning of the plants. They are biosynthesized from geranyl pyrophosphate, the ubiquitous acyclic C10 intermediate of the isoprenoid pathway [8]. They can be classified into two major groups: monoterpene hydrocarbons and oxygenated monoterpenes. The latter group includes alcohols, aldehydes, ketones, ethers and acids [9, 10]. The high biological activity of some monoterpenes against many agricultural pests include bacteria, fungi, insects, herbs, and mites, make them useful as potential alternatives to harmful synthetic pesticides as well as good lead compounds for the development of safe, effective, and fully biodegradable pesticides [11-21]. In addition, quantitative structure-activity relationships (QSAR) have not been determined, so the chemical basis for their bactericidal properties is not yet known. Developing these relationships can facilitate the design of more effective bactericidal monoterpenoids and outline the structural properties which are responsible for their biological activity [11, 22-24]. The development QSAR investigated a variety of parameters that explain the different biological and physicochemical effects and interactions between the active molecule and target site. Different physicochemical parameters include molecular weight (MW), calculated hydrophobic parameter (logarithm of partition coefficient, ClogP), molar refractivity (MR), number of valence electrons (NVE), hydrogen bond acceptor (HA), hydrogen bond donor (HD), vapour pressure (VP), and the aqueous solubility of a compound (LogS) were used as independent variables to help encode information about the important characteristics of monoterpenoids that are responsible for their bactericidal effects. Therefore, the main objective of the present work was to study the antibacterial activity of ten monoterpenes (camphene, camphor, carvone, fenchone, geraniol, limonene, linalool, menthone, menthol, and thymol) against different crop-threatening bacteria *Erwinia carotovora*, *Ralstonia solanacearum*, *Rhodococcus fascians*, and *Rhizobium radiobacter* which are responsible for important economic losses in fruit and vegetables throughout the world. The antimicrobial activity as MICs was investigated and discussed in details. Use of selected physicochemical properties, the toxicity of these compounds was then used to develop QSAR models.

MATERIALS AND METHODS

2.1. Chemicals and plant pathogenic bacteria

Ten pure monoterpenes [camphene (95%), (R)-camphor (98%), (R)-carvone (98%), (S)-fenchone (98%), geraniol (98%), (S)-limonene (96%), (R)-linalool (95%), (1R,2 S,5R)-menthol (98%), menthone (96%), and thymol (98%)], and 2,3,5,-triphenyltetrazolium chloride (TTC) were purchased from Sigma-Aldrich Co. (USA). The chemical structures of the tested monoterpenes and physicochemical descriptors used in QSAR analysis are present in Table 1. Nutrient agar (NA) was purchased from Oxoid Ltd. (Basingstoke, Hampshire, UK) and used to re-activate and propagate the tested bacteria. All of the other reagents used were of high purity grade. Microorganisms used in this work were four bacteria *Erwinia carotovora*, *Ralstonia solanacearum*, *Rhodococcus fascians*, and *Rhizobium radiobacter* which obtained from Microbiology Laboratory, Department of Plant Pathology, Faculty of Agriculture, Alexandria University, Egypt. Bacteria maintained on NA medium at 37°C.

2.2. The *in-vitro* antibacterial assay

2.2.1. Broth microdilution technique

Nutrient broth (NB) medium was used to grow the bacterial strains to a final inoculum size of 5×10^5 cfu/mL that calculated as a number of colonies × dilution factor / volume of culture plate using haemocytometer. 40 µL of serially diluted monoterpenes which dissolved in dimethyl sulfoxide (DMSO) was added to the wells of a sterile 96-well microtitre plate. Followed by the addition of 140 µL of NB medium and then 20 µL of bacterial suspension. The final volume in each well was 200 µL and the concentrations of 75, 100, 150, 200, 300, 400, 600, 800, 1000, 1200, and 1600 mg/L were tested for each compound. Control wells were prepared with culture medium, bacterial suspension only, and solvent. The contents of each well were mixed on a microplate shaker at 200 rpm for 1 min prior to incubation for 24 h at 37°C. To indicate respiratory activity the presence of color was determined after adding 10 µL/well of TTC dissolved in water (0.01%, w/v) as a chromogenic marker and incubated under appropriate cultivation conditions for 30 min in the dark [25, 26]. The absorbance was measured at 492 nm in an Ultra Microplate Reader (Robonik, PVT. LTD). Positive controls were wells with a medium and the compounds. Negative controls were wells with the growth medium, bacterial suspension and the TTC reagent. The minimum inhibitory concentration (MIC) of monoterpenes was determined as the lowest concentration where no viability was observed after 24 h on the basis of metabolic activity. All measurements of MIC values were repeated in triplicate.

2.2.2. Agar dilution technique

The *in vitro* antibacterial activity of monoterpenes was assayed using NA dilution method according to the European committee for antimicrobial susceptibility testing (EUCAST) [27] against the tested bacteria. The monoterpenes were dissolved in DMSO to obtain the main stock solution. Preliminary screening tests were performed at concentrations ranging from 100 to 3000 mg/L of each compound. For determination of MIC, different concentrations were added to NA medium immediately before it was poured into the Petri dishes at a temperature of 40-45°C. Parallel controls were maintained with DMSO mixed with NA medium. One loopful of microorganism suspensions in NB medium (≈ 6 µL) was spotted on the surface of NA medium (ten spots per plate) then incubated at 37°C for 24 h. Each concentration was tested in triplicate. The MIC was recorded in each case as the minimum concentration of compound, which inhibited the growth of tested microorganism after incubation. From the MIC observed, the intermediate concentrations between MIC values were prepared by suitable dilution of stock solution and the accurate MIC values were determined.

2.3. Quantitative structure activity relationship (QSAR) analysis

To develop QSAR, one needs to select a few descriptors from large physiochemical properties. The selected descriptors were used as independent variables and the pMIC (calculated from broth microdilution technique) was used as dependant variable to create the regression equations. Molecular weight (MW), molar refractivity (MR), topological polar surface area (tPSA), and critical volume (Cv, cm^2/mol) were computed by ChemDraw Ultra 11.0 software package. Calculated hydrophobic parameter (ClogP, logarithm of partition coefficient) was calculated by EPI Suite™ v4.11 on the basis of the work of Hansch and Fujita [28]. The hydrogen bond acceptors (HA), hydrogen bond donors (HD), and vapour pressure (Vp) were calculated by using ChemSpider (http://www.chemspider.com/). The aqueous solubility of a compound (LogS) was calculated by ALOGPS 2.1 program [29]. The number of valence electrons (NVE) was calculated by BioByte Bio-Loom program, v1.0. The stepwise multiple linear regression (MLR) analysis method was used to perform QSAR analysis by using Build QSAR software Version 2.1.0.0. The QSAR analysis was used to study the correlation between dependant variable (biological activity parameter expressed as pMIC) and independent variables include MW, ClogP, MR, NVE, HA, HD, Vp, LogS, tPSA, and Cv. The degree of correlation between the variables was justified by the correlation coefficient (*r*), the standard error of estimates (s) and the value of the ratio between regression and residual variances (*f*, Fisher's statistic). Only those parameters having good correlation coefficient and low standard error were considered to determine best equation. Regression was built using descriptor subsets containing only one of these highly correlated descriptors using Leave-one-out Cross Validation (LOOCV) method [30, 31]. MLR analysis was carried out to find out the factors responsible for variation in the biological activity. Successive regression equations were derived in which parameters are added, removed, or replaced until *r* and s values are optimized. To derive QSAR models, stepwise MLR analysis with LOOCV technique was applied to a single set of 6 compounds and the validation of the resulting models was evaluated on a test set of 4 compounds. Models with cross correlation (q^2) and *r* more than 0.90 were validated. In addition, the number of significant descriptors in the final model was also based on an overall improvement of q^2 and other the statistical data of analysis. Intercorrelation between the descriptors was checked for independence of the variables. The QSAR models with high statistical significance are reported herein.

RESULTS

3.1. Antibacterial efficiency of monoterpenes

The *in vitro* antibacterial activities of 10 monoterpenes, by broth microdilution and NA dilution techniques, against *E. carotovora*, *R. solanacearum*, *R. fascians*, and *R. radiobacter A* are presented in Tables 2, respectively. The results are presented as MIC and showed that the values obtained by broth microdilution technique were lower than that obtained by agar dilution technique which indicates that the first technique is more sensitive than the second one. The results proved that thymol and geraniol were the most effective tested monoterpenes against all tested bacteria. Thymol gave MICs 190, 170, 175, and 205 mg/L against *R. radiobacter*, *R. fascians*, *E. carotovora*, and *R. solanacearum*, respectively by broth microdilution method. However, MICs 225, 175, 200, and 210 mg/L of thymol were found against *R. radiobacter*, *R. fascians*, *E. carotovora*, and *R. solanacearum*, respectively by NA dilution technique (Table 2 and Figure 1). Geraniol showed MICs of 250, 230, 255, and 260 mg/L against *R. radiobacter*, *R. fascians*, *E. carotovora*, and *R. solanacearum* respectively by broth microdilution technique whereas MICs of 265, 250, 260, and 275 mg/L obtained against the same bacteria by NA dilution technique.

With regard to the structure-antibacterial activity relationship, thymol (an aromatic monocyclic alcohol monoterpene) and geraniol (acyclic alcohol monoterpene) (Table 1) were the most potent compounds against the

four tested bacteria among the tested compounds. Therefore, the two structurally related compounds are the most important among all the tested monoterpenoids. Camphene, carvone, fenchone, linalool, menthol, and menthone showed moderate activity while camphor and limonene were low in activity.

3.2. QSAR analysis

For QSAR analysis, the respective MIC values in mg/L of these compounds were converted to mole/L and then to -log MIC (pMIC), where MIC value is the concentration of the compound required for 100% inhibition of the growth of the microorganisms. Based on these values, statistically significant equations were generated. Different sets of equations were produced for *R. radiobacter*, *R. fascians*, *E. carotovora*, and *R. solanacearum* as follows. The various physiochemical molecular descriptors computed for investigations were MW, ClogP, MR, NVE, HA, HD, Vp, LogS, tPSA, and Cv. For *R. radiobacter*, QSAR was run for training set of 6 compounds (camphene, carvone, geraniol, linalool, menthone, and thymol) and statistical parameters like *r*, *f*, s, q^2, SPress, and SDEP were calculated. They were found statistically significant and the regression analysis was run to yield the following three models (1-3):

pMIC = + 0.0617 (± 0.0406) MW - 0.3092 (± 0.1901) MR - 0.0372 (± 0.0242) NVE + 0.9761 (± 0.5124) HD + 9.4506 (± 5.0166) ……. (1)

Where, number of compounds, n = 6; correlation coefficient, *r* = 0.999; significant, s = 0.021; Fisher test, *f* = 246.237; p = 0.0478; squired cross-validation, q^2 = 0.717; statistic of predicted residual error sum of squares, SPress = 0.356; standard deviation of prediction, SDEP = 0.159.

pMIC = + 0.4763 (± 0.3528) ClogP - 0.0621 (± 0.0266) NVE - 0.4997 (± 0.2484) Vp - 0.0025 (± 0.0041) Cv + 6.3582 (± 2.8889) ……..…… (2)

(n = 6; *r* = 1.000; s = 0.020; *f* = 281.456; p = 0.0447; q^2 = 0.328; SPress = 0.548; SDEP = 0.245).

pMIC = + 0.2680 (± 0.0449) ClogP - 0.0527 (± 0.0040) NVE + 0.0676 (± 0.0057) tPSA - 0.0047 (± 0.0007) Cv + 6.1720 (± 0.4913) …… (3)

(n = 6; *r* = 1.000; s = 0.003; *f* = 9707.743; p = 0.0076; q^2 = 0.926; SPress = 0.182; SDEP = 0.081)

Model 3 indicated the highest squired cross-validation (q^2 = 0.926) between four descriptors of ClogP, NVE, tPSA, and Cv and it was found to be the best for prediction the antibacterial activity of this series of compounds against *R. radiobacter*. This revealed that these four molecular descriptors have a significant effect on the biological activity. The parameters of ClogP and tPSA were correlated positively with the biological activity, indicating that the increase in these parameters led to increase the antibacterial activity. However, the NVE and Cv parameters correlate negatively with biological activity, indicating that the increase in these parameters led to decrease the antibacterial activity of the tested compounds.

The model 3 has a significance level as high *f* value (9707.743) and very low of s (0.003) and SDEP (0.081) compared to the other two models, demonstrate high accuracy of this model. The observed, calculated and predicted activities (pMIC) for training set of model 3 against *R. radiobacter* is presented in Table 3. The applicability of model 3 in predicting activities of external molecules or test set compounds (camphor fenchone, limonene, and menthol) is presented in Table 4. Further the plot of linear regression of experimental or observed pMIC values against the predicted pMIC values for the training set molecules also favors the model expressed by Equation 3 as shown in Figure 2A. To investigate the existence of a systemic error in developing the QSAR model, we have plotted pMIC observed against pMIC residual values for the training set molecules (Figure 2B). The propagation of the residuals on both sides of zero indicates that there is no systemic error in the development of linear regression model [32].

For *R. fascians*, variations in the biological activity of monoterpenes were analyzed using the best fit molecular descriptors (four variables) for training set of 6 monoterpenes (camphene, carvone, geraniol, linalool, menthone, and thymol) resulting the following two models (4 and 5):

pMIC = + 0.0286 (± 0.0030) MW + 0.1847 (± 0.0134) MR - 0.0420 (± 0.0022) NVE - 0.0154 (± 0.0007) Cv + 0.1561 (± 0.2921) ……..…… (4)

(n = 6; *r* = 1.000; s = 0.002; *f* = 28540.706; p = 0.0044; q^2 = 0.997; SPress = 0.035; SDEP = 0.016)

pMIC = + 0.0411 (± 0.0274) MW - 0.0409 (± 0.0223) NVE + 0.3483 (± 0.2536) HD - 0.0099 (± 0.0052) Cv + 3.7958 (± 2.6371) ……..…… (5)

(n = 6; *r* = 1.000; s = 0.019; *f* = 282.730; p = 0.0446; q^2 = 0.724; SPress = 0.340; SDEP = 0.152)

Model 4 showed the highest q^2 (0.997) between four descriptors of MW, MR, NVE, and Cv therefore, it was found to be the best model for prediction the antibacterial activity of this series of compounds against *R. fascians*. The parameters of MW and MR were correlated positively with the antibacterial activity, indicating that the increase in these parameters led to increase the antibacterial activity however, the NVE and Cv correlate negatively with biological activity. This model indicated high *f* value (28540.706) and very low of s (0.002) and SDEP (0.016) compared to model 5, demonstrate high accuracy of this model. The observed, calculated and predicted activities (pMIC) for training set of model 4 against *R. fascians* is presented in Table 5. The model was also used in predicting the antibacterial activities of external molecules or the test set molecules (camphor fenchone, limonene, and menthol) and the data are presented in Table 4. The plot of linear regression of observed pMIC against the predicted pMIC values for the training set molecules also favors the model expressed by Equation 4 as shown in Figure 3A and the plotted pMIC observed against pMIC residual values for the training set molecules is shown in Figure 3B.

The values of physiochemical molecular descriptors for monoterpenes used for QSAR analysis against *E. carotovora* are indicated in Equation 3 as follows: The values of molecular descriptors for training set monoterpenes (camphene, carvone, geraniol, linalool, menthone, and thymol) were used for QSAR analysis against *E. carotovora* and the results showed two fit models (6 and 7) as follows:

pMIC = + 0.0222 (± 0.0249) MW + 0.2264 (± 0.1110) MR - 0.0324 (± 0.0183) NVE - 0.0154 (± 0.0061) Cv - 1.4836 (± 2.4259) …….…… (6)

(n = 6; r = 1.000; s = 0.016; f = 405.745; p = 0.0372; q^2 = 0.796; SPress = 0.289; SDEP = 0.129)

pMIC = + 0.0376 (± 0.0143) MW - 0.0311 (± 0.0117) NVE + 0.4278 (± 0.1324) HD - 0.0086 (± 0.0027) Cv + 2.9811 (± 1.3773) …….…… (7)

(n = 6; r = 1.000; s = 0.010; f = 1017.230; p = 0.0235; q^2 = 0.923; SPress = 0.177; SDEP = 0.079)

From this analysis, model 7 was the best fit model against *E. carotovora* that showed the highest q^2 (0.923) between four descriptors (MW, NVE, HD, and Cv). It has also a highest *f* value (1017.230) and very low of s (0.010) and SDEP (0.079) compared to model 6 (f = 405.745, s = .016, and SDEP = 0.129). MW and HD were correlated positively with the antibacterial activity and high coefficient constant was obtained with HD (0.4278). However, NVE and Cv correlate negatively with the biological activity. The observed, calculated and predicted activities (pMIC) for training set (6 molecules) of model 7 against *E. carotovora* is indicated in Table 6. This model was applied to predict the antibacterial activities of the external test set compounds (camphor fenchone, limonene, and menthol) and the results are presented in Table 4. In addition, the observed pMIC versus calculated pMIC values according to model 7 were plotted in Figure 4A and the plotted pMIC observed against pMIC residual values for the training set molecules is shown in Figure 4B.

QSAR analysis for *R. solanacearum*, variations in the biological activity of the tested monoterpenes were analyzed using the best fit molecular descriptors (four variables) for training set of 6 compounds (camphene, carvone, geraniol, linalool, menthone, and thymol) and the correlations are indicated the following three models (8, 9, and 10):

pMIC = + 0.6111 (± 0.2132) ClogP - 0.0141 (± 0.0490) MR - 0.0660 (± 0.0184) NVE - 0.5644 (± 0.1780) Vp + 5.5487 (± 2.7975) …….…… (8)

(n = 6; r = 1.000; s = 0.013; f = 702.831; p = 0.0283; q^2 = 0.825; SPress = 0.282; SDEP = 0.126)

pMIC = + 0.4469 (± 0.2849) ClogP - 0.0680 (± 0.0989) MR - 0.0599 (± 0.0286) NVE + 0.0896 (± 0.0476) tPSA + 6.4346 (± 4.9666) …….…… (9)

(n = 6; r = 0.999; s = 0.021; f = 247.707; p = 0.0476; q^2 = 0.448; SPress = 0.501; SDEP = 0.224)

pMIC = + 0.5808 (± 0.0379) ClogP - 0.0644 (± 0.0029) NVE - 0.5387 (± 0.0267) Vp - 0.0008 (± 0.0004) Cv + 5.2764 (± 0.3103) ……..… (10)

(n = 6; r = 1.000; s = 0.002; f = 24891.562; p = 0.0048; q^2 = 0.992; SPress = 0.059; SDEP = 0.026)

As we can see her, the model 10 showed the highest q^2 (0.992) between four descriptors of ClogP, NVE, Vp, and Cv and it was found to be the best one compared to models 8 and 9 for prediction the antibacterial activity of this series of compounds against *R. solanacearum*. It has a significance level as high *f* value (24891.562) and very low of s (0.002) and SDEP (0.026) compared to the other two models, demonstrate high accuracy of this model. ClogP was correlated positively with the biological activity in all three obtained models, indicating that the increase of it led to increase the antibacterial activity. However, the NVE, Vp, and Cv parameters in model 10 correlate negatively with

biological activity, indicating that the increase in these parameters led to decrease the antibacterial activity of the tested compounds. The observed, calculated and predicted activities (pMIC) for training set of model 10 against *R. solanacearum* is presented in Table 7. The applicability of this model for prediction the activities of external test set compounds (camphor fenchone, limonene, and menthol) are presented in Table 4. In addition, the plot of linear regression of experimental or observed pMIC values against the predicted pMIC values for the training set molecules also by model 10 is shown in Figure 5A and the plotted pMIC observed against pMIC residual values for the training set molecules is shown in Figure 5B.

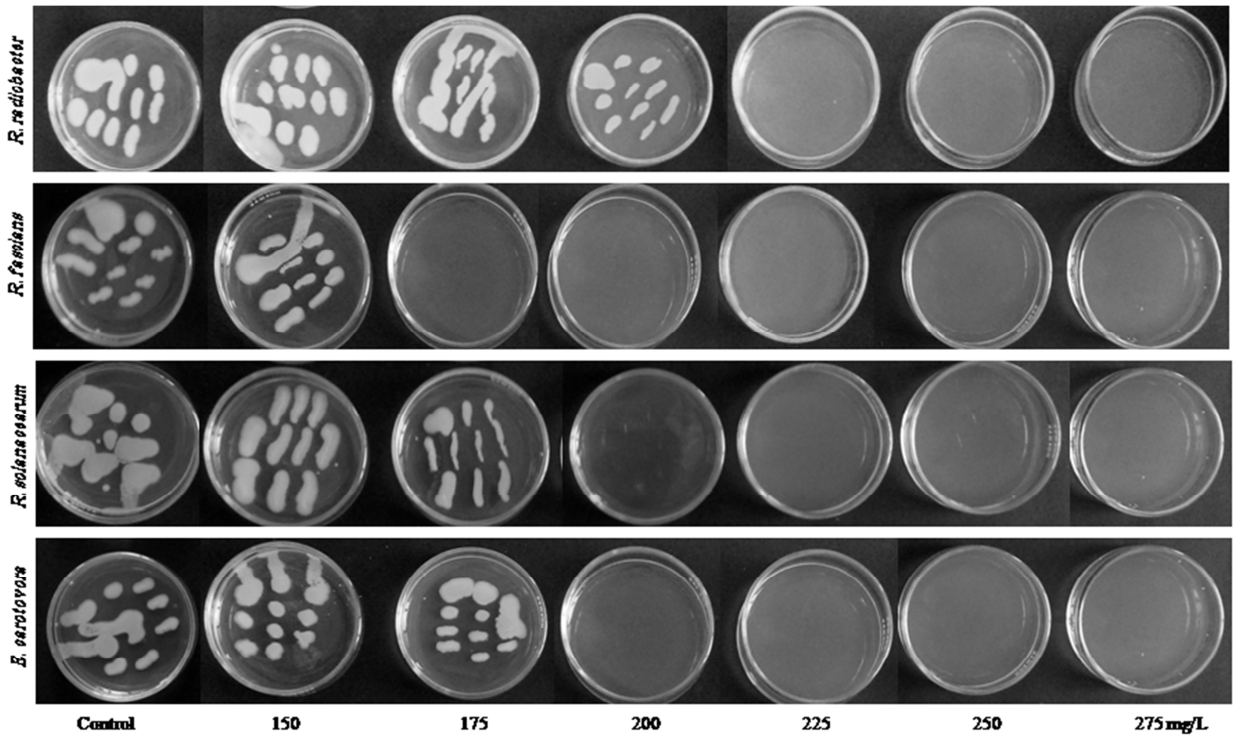


Figure 1. The *in vitro* growth of *E. carotovora*, *R. solanacearum*, *R. fascians*, and *R. radiobacter* in NA plates incorporated with 0, 150, 175, 200, 225, and 275 mg/L of thymol

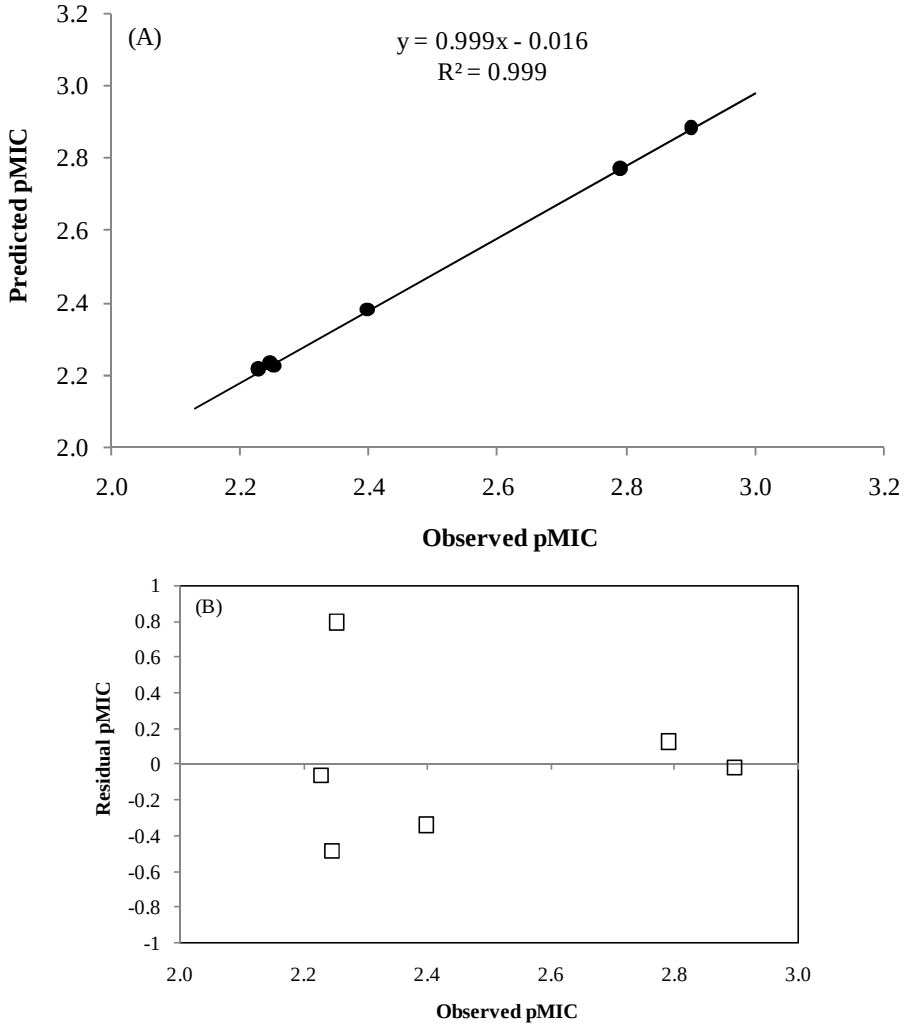
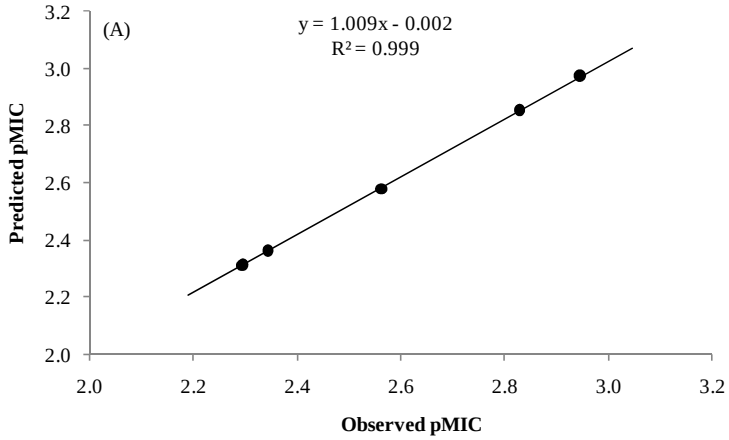


Figure 2. A: Plot of predicted pMIC of natural monoterpenes (n = 6) activity against *R. radiobacter* by broth microdilution technique versus the experimental pMIC values for the linear regression analysis developed by model 3. B: Plot of residual pMIC against the experimental pMIC values



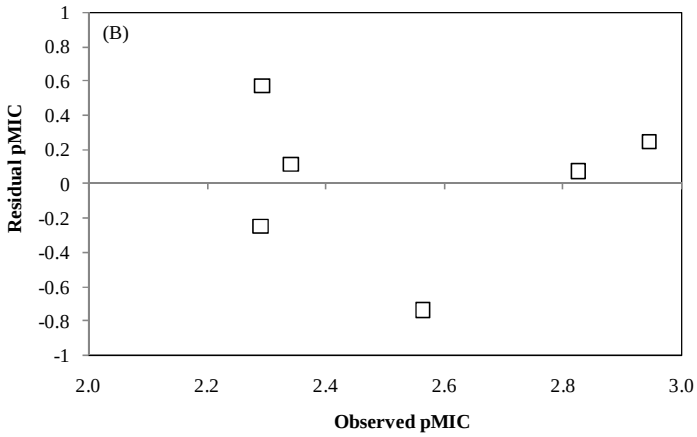
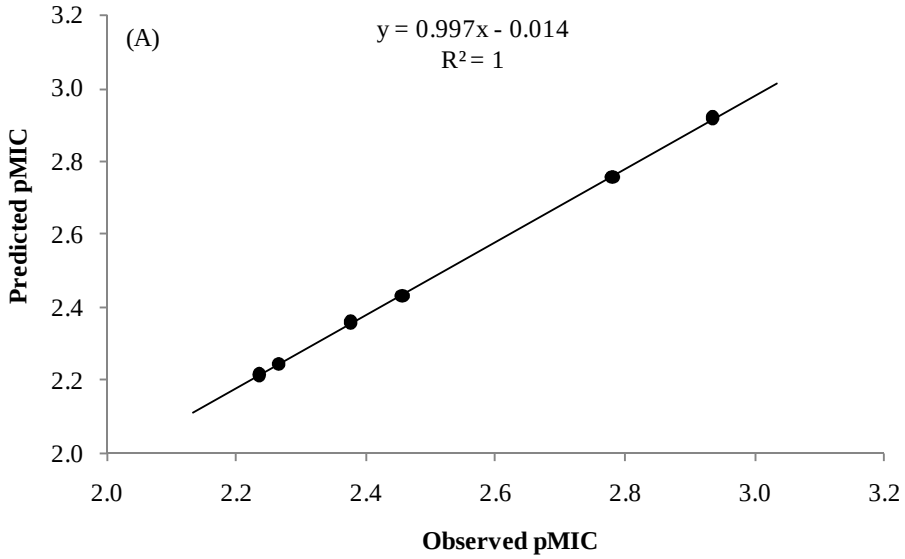


Figure 3. A: Plot of predicted pMIC of natural monoterpenes (n = 6) activity against *R. fascians* by broth microdilution technique versus the experimental pMIC values for the linear regression analysis developed by model 4. B: Plot of residual pMIC against the experimental pMIC values



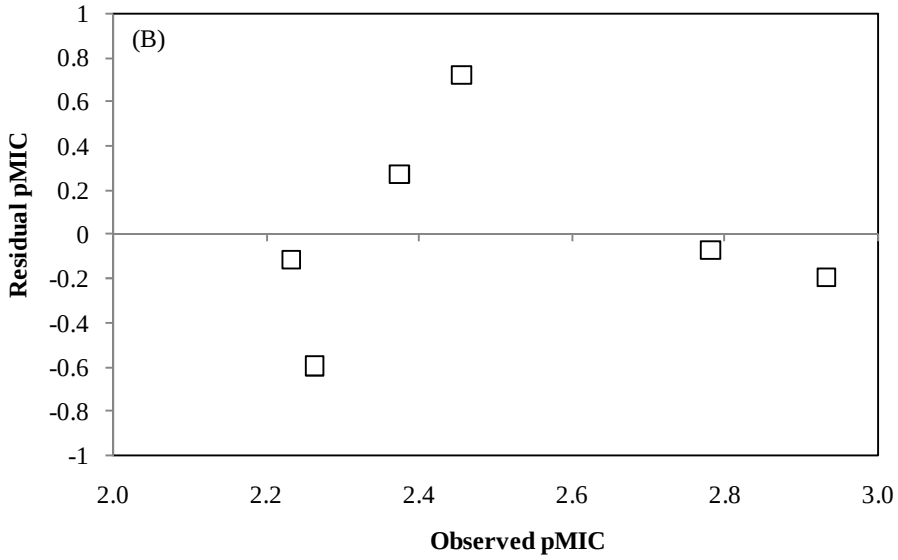
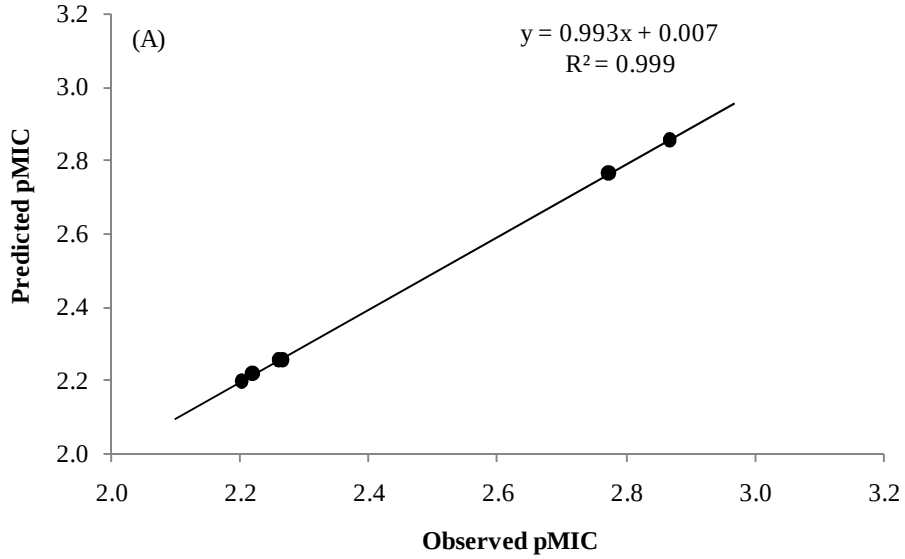


Figure 4. A: Plot of predicted pMIC of natural monoterpenes (n = 6) activity against *E. carotovora* by broth microdilution technique versus the experimental pMIC values for the linear regression analysis developed by model 7. B: Plot of residual pMIC against the experimental pMIC values



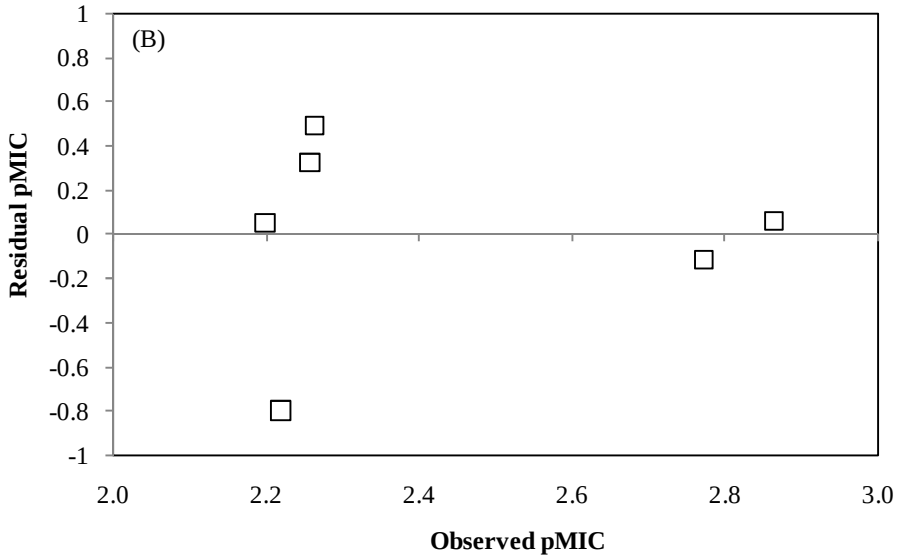
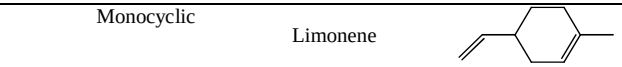
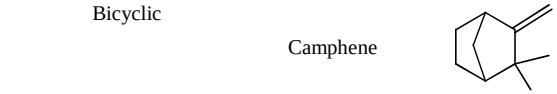
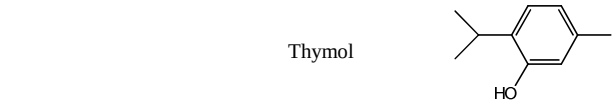


Figure 5. A: Plot of predicted pMIC of natural monoterpenes (n = 6) activity against *R. solanacearum* by broth microdilution technique versus the experimental pMIC values for the linear regression analysis developed by model 10. B: Plot of residual pMIC against the experimental pMIC values

Table 1. Chemical structure of tested monoterpenes and physicochemical descriptors used in QSAR analysis

Type	Chemical class	Common name	Chemical structure	physicochemical descriptors									
				MW	**ClogP**	**MR**	**NVE**	**HA**	**HD**	**Vp**	**LogS**	**tPSA**	**Cv**
Hydrocarbon monoterpenes	Monocyclic	Limonene		136.23	4.7	43.8	56	0	0	3.38	-3.34	0	482.5
	Bicyclic	Camphene		150.22	2.2	45.5	60	1	0	0.066	-2.11	17	503.5
Oxygenated monoterpenes (Monoterpenoids)	Acyclic alcohol	Geraniol		154.25	1.8	49.7	48	1	1	0.013	-2.05	20	576.5
		Linalool		154.25	2.75	49.5	64	1	1	0.091	-2.51	20	565.5
	Monocyclic alcohol	Menthol		154.25	2.83	46.4	64	1	0	0.3	-2.77	17.07	528.5
		Thymol		150.22	3.2	47.1	60	1	1	0.038	-2.37	20	497.5
	Monocyclic ketones	Carvone		136.23	4.7	43.8	56	0	0	3.38	-3.34	0	482.5
		Menthone		150.22	2.2	45.5	60	1	0	0.066	-2.11	17	503.5
	Bicyclic ketones	Camphor		154.25	1.8	49.7	48	1	1	0.013	-2.05	20	576.5
		Fenchone		154.25	2.75	49.5	64	1	1	0.091	-2.51	20	565.5

***MW**: molecular weight, **ClogP**: Calculated hydrophobic parameter (logarithm of partition coefficient), **MR**: molar refractivity (cm^3/mol), **NVE**: Number of valence electrons, **HA**: hydrogen bond acceptor, **HD**: hydrogen bond donor, **Vp**: vapour pressure (mmHg at 25°C), **LogS**: the aqueous solubility of a compound, **tPSA**: Topological Polar Surface Area, and **Cv**: Critical volume (cm^2/mol)*

Table 2. *In vitro* antibacterial activity of natural monoterpenes against *E. carotovora*, *R. solanacearum*, *R. fascians*, and *R. radiobacter* by broth microdilution and NA dilution techniques

Monoterpenes	MIC (mg/L)							
	Broth microdilution technique				NA dilution technique			
	R. radiobacter	*R. fascians*	*E. carotovora*	*R. solanacearum*	*R. radiobacter*	*R. fascians*	*E. carotovora*	*R. solanacearum*
Camphene	805	620	795	860	815	700	825	875
Camphor	1150	1170	940	1095	1350	1310	1000	1125
Carvone	600	410	525	830	740	525	540	840
Fenchone	940	840	865	870	925	1025	1050	1000
Geraniol	250	230	255	260	265	250	260	275
Limonene	1060	850	840	1050	1275	1000	900	1125
Linalool	875	790	650	840	925	800	675	850
Menthol	515	420	400	520	540	450	500	525
Menthone	860	785	840	930	975	825	850	950
Thymol	190	170	175	205	225	175	200	210

MIC is a minimum inhibitory concentration value obtained for each microorganism.

Table 3. Training set activity by using QSAR model 3 against *R. radiobacter*

Monoterpenes	Observed pMIC	Calculated pMIC	Predicted pMIC
Camphene	2.228	2.230	2.213
Carvone	2.399	2.401	2.382
Geraniol	2.790	2.790	2.767
Linalool	2.246	2.252	2.230
Menthone	2.254	2.247	2.228
Thymol	2.898	2.900	2.881

Table 4. Test set activities by using QSAR models against *R. radiobacter*, *R. fascians*, *R. solanacearum*, and *E. carotovora*

Monoterpenes	Model 3 for *R. radiobacter*		Model 4 for *R. fascians*		Model 7 for *E. carotovora*		Model 10 for *R. solanacearum*	
	Observed pMIC	Predicted pMIC	Observed pMIC	Predicted pMIC	Observed pMIC	Predicted pMIC	Observed pMIC	Predicted pMIC
Camphor	2.122	2.272	2.114	2.353	2.209	2.447	2.143	2.026
Fenchone	2.209	2.119	2.258	2.353	2.245	2.447	2.243	1.566
Limonene	2.109	2.053	2.205	2.440	2.210	2.092	2.113	2.970
Menthol	2.482	2.376	2.571	2.374	2.592	2.592	2.478	2.453

Table 5. Training set activity by using QSAR model 4 against *R. fascians*

Monoterpenes	Observed pMIC	Calculated pMIC	Predicted pMIC
Camphene	2.342	2.340	2.360
Carvone	2.564	2.561	2.582
Geraniol	2.826	2.830	2.853
Linalool	2.291	2.290	2.314
Menthone	2.293	2.289	2.311
Thymol	2.946	2.950	2.970

Table 6. Training set activity by using QSAR model 7 against *E. carotovora*

Monoterpenes	Observed pMIC	Calculated pMIC	Predicted pMIC
Camphene	2.234	2.231	2.212
Carvone	2.457	2.453	2.433
Geraniol	2.782	2.781	2.758
Linalool	2.375	2.377	2.355
Menthone	2.264	2.266	2.245
Thymol	2.934	2.932	2.913

Table 7. Training set activity by using QSAR model 10 against *R. solanacearum*

Monoterpenes	Observed pMIC	Calculated pMIC	Predicted pMIC
Camphene	2.200	2.200	2.193
Carvone	2.258	2.259	2.252
Geraniol	2.773	2.770	2.762
Linalool	2.264	2.259	2.251
Menthone	2.220	2.222	2.214
Thymol	2.865	2.860	2.853

DISCUSSION

The current study investigate the antibacterial activity of natural monoterpenes against four plant pathogenic bacteria *E. carotovora*, *R. solanacearum*, *R. fascians*, and *R. radiobacter* by broth microdilution and NA dilution techniques. The results obtained coincide with the results of Penalver and co-authors, who indicated that the higher inhibitory capacity for microorganisms was observed in the essential oils with a higher percentage of phenolic components (carvacrol and thymol) [33]. Also, El-Zemity and co-authors reported that the antibacterial activity was strongly associated with monoterpenic phenols include thymol, chlorothymol, and carvacrol [34]. Marei and co-authiors added that the thymol was the most potent antifungal compound among twelve monoterpenes (camphene, (R)-camphor, (R)-carvone, 1,8-cineole, cuminaldehyde, (S)-fenchone, geraniol, (S)-limonene, (R)-linalool, (1R,2S,5R)-menthol, myrcene and thymol) against four plant pathogenic fungi *Rhizoctonia solani*, *Fusarium oxysporum*, *Penecillium digitatum*, and *Asperigallus niger* by using mycelial growth inhibitory technique with EC_{50} of 33.50, 50.35, 20.14 and 23.80 mg/L, respectively [35]. It has been also reported that thymol completely inhibited mycelial growth of 17 phytopathogenic fungi, including *R. solani* and *F. oxysporum* [36]. In addition, carvone had a potential to control potato sprout and it had promising antifungal activity against other potato storage diseases *F. sulphureum*, *Phoma exigua*, and *Helminthosporium solani* [37]. Monoterpenes are highly hydrophobic substances present in essential oils. They cover a wide spectrum of biological effects microorganism cells involves cytoplasm granulation, cytoplasmic membrane rupturing and inactivation and/or synthesis inhibition of intracellular and extracellular enzymes [12, 38-40]. Moreover, they induced of membrane fatty acids composition of microbial cells [12, 41-43]. Thus, the antibacterial activity of monoterpenoids in the present study may be due to their interaction with the membrane of bacterial cells. Such inhibition is due to interaction with the phospholipid bilayer of the cell membrane, causing increased permeability and loss of cellular constituents [39, 40, 44-48]. Trombetta and co-authors studied the mechanism of action of three monoterpenes [linalyl acetate, (+) menthol, and thymol] against the gram-positive bacterium *Staphylococcus aureus* and the gram-negative bacterium *Escherichia coli* and they reported that the antimicrobial effect of (+) menthol, thymol, and linalyl acetate may be due, at least partially, to a perturbation of the lipid fraction of bacterial plasma membranes, resulting in alterations of membrane permeability and leakage of intracellular materials [39]. However, they reported that the (+) menthol was more effective than thymol against *E. coli*, while thymol was more toxic for *S. aureus* [39] as the gram-negative bacteria outer membrane presented a strong negative charge conferred by lipopolysaccharide [44].

In addition, the QSAR results showed ten statistically significant models, which predicted the antibacterial activity in lineal equations. The models obtained through the QSAR analysis gave a better prediction of the antibacterial activity and the descriptors include MW, ClogP, MR, HD, and tPSA were the major factors responsible for positively affecting the antibacterial activity however, NVE and Cv showed negative correlation. The molecular descriptors of MW, ClogP, MR, NVE, HD, tPSA, and Cv are very useful parameter for prediction of the antibacterial activity of the tested monoterpenes against plant pathogenic bacteria. MW, ClogP, MR, HD, and tPSA were significantly the highest correlation found with all tested bacteria and were correlated positively with the biological activity in all the best models (3, 4, 7 and 10), indicating that the increase of these parameters in the monoterpenes led to enhance the antibacterial activity. However, the NVE and Cv were correlated negatively with biological activity in all models, indicating that the increase of these descriptors led to a decrease of the activity. This relationship might be due to the electrostatic interaction of these compounds to a receptor, and as electron accessibility for the monoterpenoids molecules increases, binding affinity also increases. A positive contribution of MR with activity against *Escherichia coli* was also reported by Gupta and co-authors [49]. Recent literature reveals that the QSAR has been applied to describe the relationship between narrow range of biological activity and physiochemical properties of the molecules. When biological activity data lie in a narrow range, the presence of minimum standard deviation of the biological activity justifies its use in QSAR studies [11, 24, 50-52]. The minimum standard deviation (models 3, 4, 7, and 10) observed in the antimicrobial activity data justifies its use in QSAR studies.

In conclusion, to our knowledge, this is the first contribution concerning QSAR study activity of natural monoterpenes against plant bacteria. The QSAR models could be used in the future to develop new effective alternative antibacterial agents, as well as contributing to a better understanding of their mechanism of action. According to the obtained results, the tested monoterpenes especially geraniol and thymol potentially might be used as potential environmentally friendly products and as safe alternatives to harmful synthetic pesticides to protect the crops from infection. However, formulating of such compounds is essential for commercial uses of the pesticidal monoterpenes with further *in vivo* studies are essentially needed. Such formulations can be used in organic and conventional agricultural systems if the formulations are improved for foliar application.

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Conflict of interests
The authors declare that there is no conflict of interests regarding the publication of this paper.

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