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An *in silico* study into the bioacaricide power of the Algerian argan tree against *Varroa destructor*

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Varroa destructor is the parasitic mite of the honeybee, *Apis mellifera*. It presents a major threat to the health of bees and to the quality and quantity of honey. *Varroa destructor* have increased their resistance to acaricides; consequently, the mites or their vector viruses become more virulent. Infested colonies, commonly referred to as 'mite bombs', facilitate the dispersal of mites and transmission of disease to stronger and healthier colonies. Acaricides are the most used means of control, although the use of these chemical products has a negative impact on the health of bees, the quality of honey, human health, and the environment. The argan tree of the genus *Argania* is a tropical tree of the Sapotaceae family. This plant is very important economically. The argan tree is the source of biologically active and edible oil. In this context, we aim to test the bioacaricide power of the argan tree to fight the *V. destructor* by molecular modelling methods.

Keywords: *Varroa destructor*, *Apis mellifera,* acaricide, *Argania*, bioiacaricide, molecular modelling

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INTRODUCTION

Pollinators have an essential role in the environment. The safety of our food, particularly vegetable, fruit, oilseed, and protein crop, depends on animal pollination. Therefore, the honeybee plays a very important economic role, not only in relation to its various exploited products such as honey, pollen, and royal jelly (Mekaoui, 2018) but also for its role in maintaining the diversity of plants and our food resources (Rosenkranz et al., 2010).

Varroa destructor, or the Varroa mite, the parasitic mite is considered a major pathogenic threat to the honeybee *Apis mellifera*. It is responsible for varroosis, a disease that causes the weakening and loss of colonies, and for numerous damages at the individual and colony level due to the association of the parasite with several bee viruses (Rosenkranz et al., 2010). Since the appearance of this disease, several chemical molecules have been available on the market. But the misuse of these products generates multiple drawbacks: residues in hive products, possible toxicity to bees, and the development of resistance to certain acaricidal molecules in mites (Elzen et al., 2000; Sammataro et al., 2005).

The argan tree *A. spinosa*, the only representative of the tropical family of Sapotaceae in Morocco and Algeria, is characteristic of the Sahara steppe zone (Chevalier, 1953). It protects the soil from desertification and erosion (Alados, El Aich, 2008). This tree is very important economically due to the possibility of using all the parts of the tree (oil, wood) and the uses of its parts (firewood, fodder) (Aboughe-Angone et al., 2008) (El Aich et al., 2007). *A. spinosa* is a source of biologically active and edible oil from its fruits (Stussi et al., 2005). Its oil is used in the culinary, cosmetic and traditional medicine fields (Charrouf, 2002). It has nutritional, phytochemical (Charrouf, 1984) and pharmacological qualities such as antioxidant (Cherki et al., 2005), antiproliferative (Bennani et al., 2007), cardioprotective (Charrouf et al., 2007), and hypolipidemic (Drissi et al., 2004). It is an oil rich in essential polyunsaturated fatty acids, a source of oleic acid and linoleic acid (Rahmani, 2005). It is rich in minor and noble compounds such as tocopherols, polyphenols, sterols, carotenoids, xanthophylls, squalene (Khallouki et al., 2005) and saponins (Guillaume and Charrouf, 2005) which explains its versatility and its efficiency.

The objective of this work is to theoretically test the bioacaricide power of *A. spinosa* for the control of *V. destructor* by molecular modelling methods.

MATERIAL AND METHODS

Preparation of inhibitors. The present work consists in studying the interaction and the degree of affinity between two proteins of the Varroa mite: cytochrome c oxidase and acetylcholinesterase with a series of molecules extracted from the essential oils of the fruit part of *A. spinosa* to have the most stable complex that means the lowest energy level. The plant molecules that are the subject of this study are: oleic acid, palmitic acid, gamma-tocopherol, Schottenol, palmitodiolene (POO). They were chosen according to the highest yield, which is expressed by the quantity of oil (ml) obtained from 100 g of dry plant matter (Akrout, 2004). Their chemical structure is presented in Tables 1, 2, and 3. We downloaded the chemical structure of the inhibitors from the PubChem Drugbank database and performed molecular docking using the PyRx Autodock Vina software (v. 0.8).

We then downloaded and performed the docking with synthetic molecules (Amitraz, Coumaphos, Tau-fluvalinate) and molecules naturally present in honey (formic acid, oxalic acid, thymol) to establish a comparison between the three types of molecules (plant molecules, synthetic, and naturally present in honey) to choose the best Varroa mite inhibitor.

Preparation of the protein. We downloaded the cytochrome c oxidase protein under the access code 7AU6 from the Protein Data

Table 1. **Chemical structures of plant molecules extracted from the *A. spinosa* tree (*DrugBank – PubChem Data Source*)**

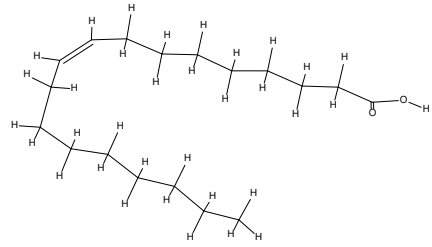
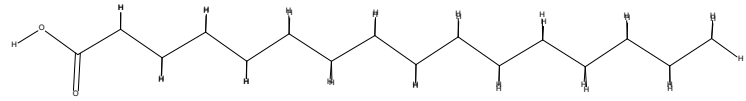
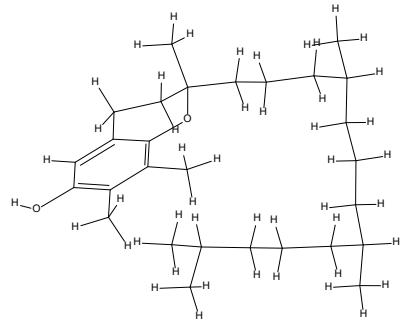
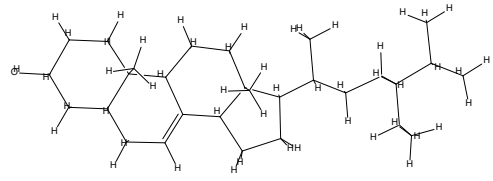
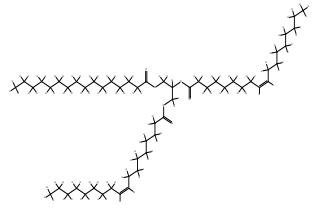
Chemical compound	Chemical structure
Oleic acid	
Palmitic acid	
Gamma-tocopherol	
Schottenol	
Palmito-diolene (POO)	

Table 2. **Chemical structure of synthetic molecules (*DrugBank – PubChem Data Source*)**

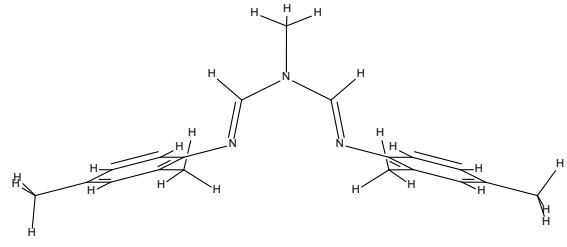
Chemical compound	Chemical structure
Amitraz	

Table 2. **(Continued)**

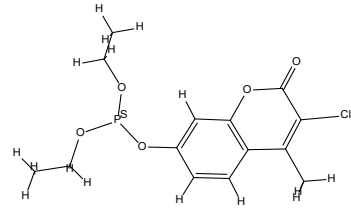
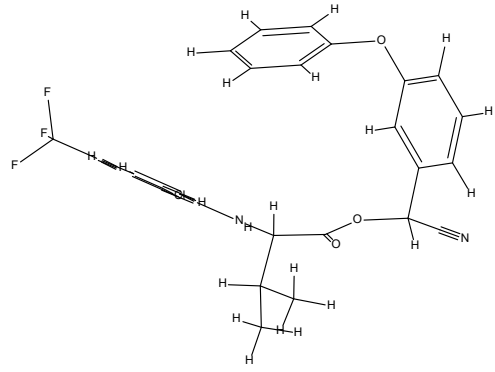
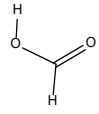
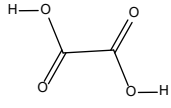
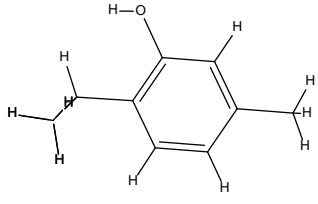
Chemical compound	Chemical structure
Coumaphos	
Tau-fluvalinate	

Table 3. **Chemical structure of molecules naturally present in honey (*DrugBank – PubChem Data Source*)**

Chemical compound	Chemical structure
Formic acid	
Oxalic acid	
Thymol	

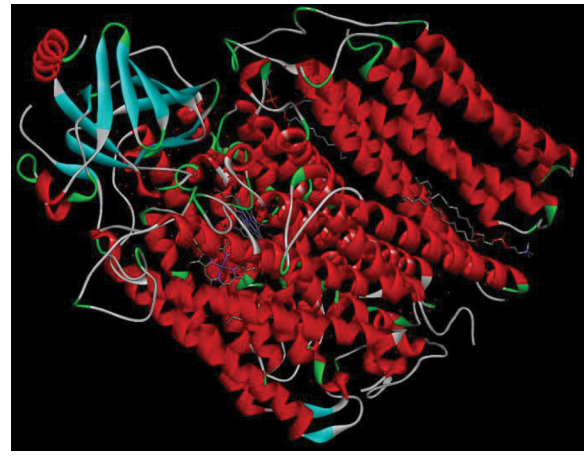


Fig. 1. Three-dimensional structure of cytochrome c oxidase protein under the access code 7AU6 (Protein Data Bank)

Bank with the resolution of 2.40 Å; composed of four chains and 1180 amino acids in total. The three-dimensional structure is shown in Fig. 1.

Thus, we downloaded the acetylcholinesterase protein under the access code 1EEA with the resolution of 4.50 Å, composed of a single chain and 534 amino acids. The three-dimensional structure is shown in Fig. 2.

Molecular docking. We used molecular modelling method in this work. It is a bioinformatics tool that allows the study of biological phenomena at the atomic scale. Molecular modelling is increasingly used to study chemical reactions and protein dynamics (Hercend, 2012). Its purpose is to predict the structure

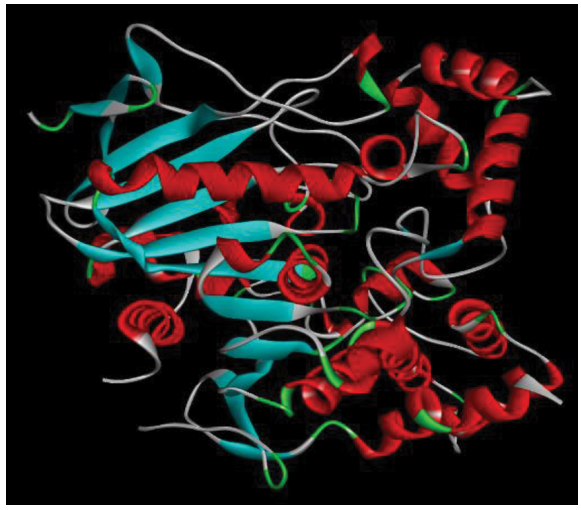


Fig. 2. Three-dimensional structure of the protein acetylcholinesterase under the accession code 1EEA (Protein Data Bank)

and reactivity of molecules (Debord). The principle of molecular modelling consists of specifying, from calculations, the position of atoms in space and calculating the energy of the structure generated. The closest possible representation to reality will correspond to a lower energy structure (Paugam, 2008). Molecular modelling involves theoretical computational methods, including molecular mechanics (MM) and molecular docking.

Simulation of molecular dynamics. Simulations of molecular dynamics were performed using Desmond at 100 ns to investigate the binding conformational stability of the protein-ligand complex. The stability of the protein-ligand complex was observed to be maintained during the whole 100 ns simulations for compounds based on RMSD (root mean square deviation) shows the stability of the docked complex, RMSF (root mean square fluctuation) indicates the conformational flexibility of the complex, and hydrogen bond interactions (Bowers 2006). The details of the procedure are available elsewhere (El Khatabi 2022).

RESULTS

Molecular docking

We performed molecular docking with the PyRx Autodock Vina software (v. 0.8) between the cytochrome c oxidase protein under the access code 7AU6 and the eleven inhibitors; then, between the acetylcholinesterase protein under the access code 1EEA and the same inhibitors (Table 4).

Table 4. **Molecular docking results**

Type of molecules	Proteins/ligands	7AU6 (cytochrome c oxidase) (Kcal/mol)	1EEA (acetylcholinesterase) (Kcal/mol)
Molecules naturally present in honey	Formic acid	–2.9	–3.1
	Oxalic acid	–4.0	–4.0
	Thymol	**–7.2**	**–6.6**
Vegetal molecules	Oleic acid	–7.5	–6.8
	Palmitic acid	–6.6	–6.0
	Gamma-tocopherol	–10.4	–8.9
	Schottenol	**–11.7**	**–11.4**
	Palmito-diolene (POO)	–7.0	–7.0
Synthetic molecules	Amitraz	–10.5	–10.1
	Coumaphos	–6.1	–8.4
	Tau-fluvalinate	**–11.3**	**–11.9**

We observed that the 7AU6-schottenol complex released an energy of –11.7 kcal/mol and the 7AU6-Tau-fluvalinate complex released an energy of –11.3 kcal/mol. So, the 7AU6-schottenol complex is the most stable since it represents a better affinity with the lowest energy level compared to the others and we note that this plant molecule is the best inhibitor compared to the other types of molecules. On the other hand, the 1EEA-schottenol complex released an energy of –11.4 kcal/mol which is close to the energy released by the 1EAA-Tau-fluvalinate complex which is –11.9 kcal/mol. Thus, schottenol inhibits the protein acetylcholinesterase as well.

Figs 3 and 4 represent the pose of the plant molecule schottenol in the enzymatic cavity of each of the two proteins.

Molecular dynamics simulation

Molecular dynamics was performed on the top hit which contains high energy bonds. Projected conformational changes from the original structure were shown by RMSD. Structural stability, atomic mobility, and flexibility residues at the time of protein-hit interaction were expressed by root mean square fluctuation (RMSF) values. The RMSD values of the protein-ligand complex are presented in Fig. 5. The RMSD of the complex showed a deviation of about 2 Å up to 15 ns, then there was no significant fluctuation, and the simulation converged. It indicates the stability of the protein-ligand complex (Fig. 5) for RMSF, there was a fluctuation of approximately 3 Å from GLU 68 to CYS 80, and the remaining structure was stable comparatively and there was not much fluctuation where

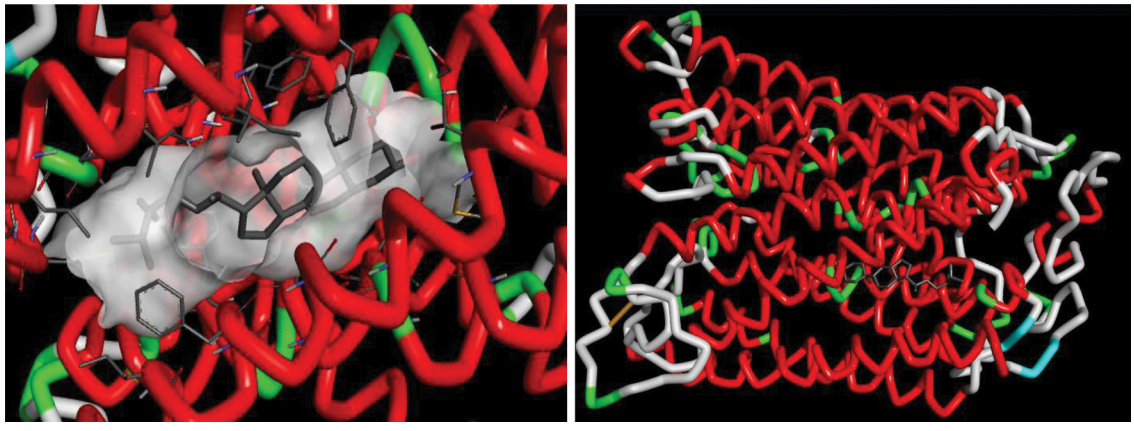


Fig. 3. The 7AU6-schottenol complex

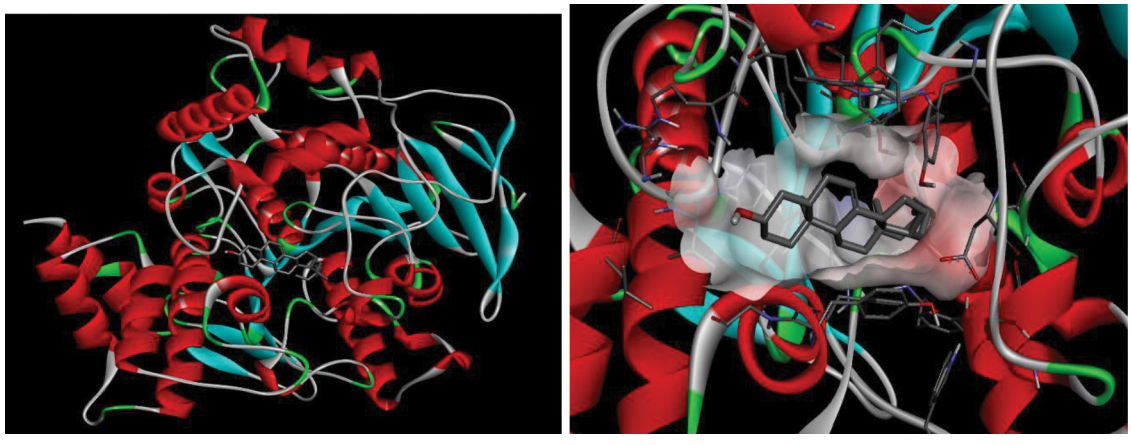


Fig. 4. The 1EEA-schottenol complex

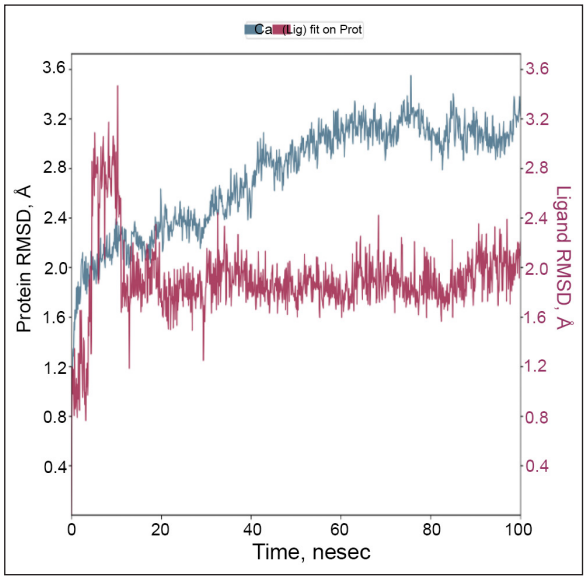


Fig. 5. The RMSD plot of protein-ligand complex

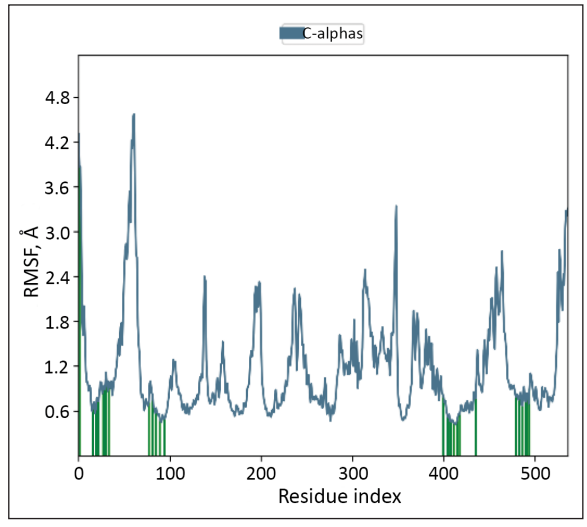


Fig. 6. The RMAF plot of the protein-ligand complex

ligand made contacts with protein (Fig. 6). The ligand showed significant different types of intermolecular interactions during the entire simulation, including hydrogen bonds, ionic, water bridges, and hydrophobic. The residues participating in these interactions include PHE 18, ILE 33, LEU 36, PHE 37, SER 46, VAL 47, THR 50, HIS 94, MET 98, VAL 102, ALA 106, PHE 111, MET 416, SER 417, VAL 421, ILE 424, PHE 425, VAL 428, ILE 432, MET 435, MET 452, SER 496, PHE 500, PHE 503, VAL 508, and LEU 511 (Fig. 7).

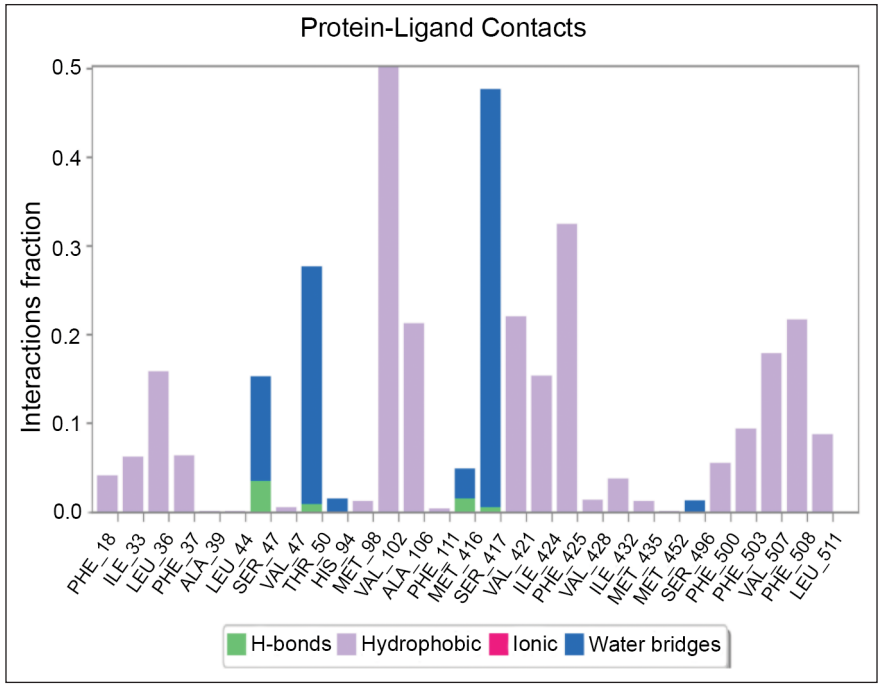


Fig. 7. Protein-ligand contacts

DISCUSSION

This paper presents the results of molecular docking and dynamic simulation between proteins of *V. destructor* and molecules extracted from the essential oils of the fruit part of *Argania spinosa* in the first time. On the other hand, it presents the results of molecular docking and dynamic simulation between proteins of *V. destructor* and synthetic molecules, then, between proteins of *V. destructor* and molecules naturally present in honey. *V. destructor* is still a major threat for apiculture during its long history (Rosenkranz et al., 2010). It causes damage to the morphology and physiology of bees. Glinski and Jarosz (1984) showed in their studies a reduction of the content of total proteins in haemolymph of drone brood parasitised by Varroa mite and the alterations were related to the intensity of the invasion.

V. destructor is also a disease vector (Gliński, Jarosz, 1992). Elzen et al. (2000) proved that Varroa mites are not only resistant to fluvalinate, but also to amitraz. Coumaphos was effective against these resistant mites. Sammataro et al. (2005) reported resistance of Varroa mite to all three acaricides (amitraz, coumaphos, and tau-fluvinate). Vilarem et al. (2021) pointed out the necessity to find a sustainable solution for honeybees and in such a way that Varroa mites do not develop any resistance. In their research results, Elzen et al. (2001) showed the efficiency of the biological activity of grapefruit leaf burning residue extract for the detachment of Varroa mite from honeybees, and grapefruit leaf burning residue had any negative side effect on the hive.

Formic acid, oxalic acid, and thymol are molecules naturally present in honey. The results of molecular docking were not too good compared with plant and synthetic molecules. The synthetic molecules (amitraz, coumaphos, and tau-fluvinate) gave a good score, especially tau-fluvinate with cytochrome c oxidase (–11.3 kcal/mol) and acetylcholenisterase (–11.9 kcal/mol). Synthetic molecules are toxic for the hive; they have a negative impact on the health of bees, the quality and quantity of honey, and therefore on human health. Our results of molecular docking show that the difference between the score given by plant molecules and that given by acaricides molecules is not big. The score given by oleic acid, palmitic acid and palmito-diolene (POO) is close to the values given by coumaphos. Amitraz and gamma-tocopherol have practically the same values; and we note the same for tau-fluvinate and schettenol. Schettenol gave good results and it has many advantages: a small size and a minimal weight compared to tau fluvinate (DrugBank – PubChem Data Source). Finally, schottenol is a biomolecule that has no negative impact on the hive and on bee health; due to that, we can propose that *A. spinosa* has a bioacaricide power to control Varroa mites.

CONCLUSIONS

Our work aimed to test theoretically the bioacaricide power of *A. spinosa* for the control of the parasitic mite *V. destructor* by molecular modelling methods, establishing a comparison between these plant molecules and a series of molecules frequently used by beekeepers.

Molecular docking results showed that the two complexes 7AU6–schottenol complex and 1EEA–schottenol complex represent a better affinity with the lowest energy level compared to the other complexes.

The molecular dynamics results show that the complexes are stable and they have formed multiple types of bonds.

Therefore, we propose the schottenol molecule extracted from essential oils of *A. spinosa* as a possible new inhibitor of *V. destructor* and confirm the bioacaricide power of this tree for the control of this parasite of honeybees.

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ARGANO MEDŽIO KAIP BIOAKARICIDO POVEIKIO *VARROA DESTRUCTOR* ERKĖMS TYRIMAS *IN SILICO* (ALŽYRAS)

Santrauka

Varroa destructor yra naminių bičių (*Apis mellifera*) parazitinė erkė, didžiausią grėsmę kelianti bičių būklei. Didėjantis *Varroa destructor* populiacijos atsparumas akaricidams rodo, kad erkės ar jų pernešami virusai tampa vis labiau virulentiški. Užsikrėtusios kolonijos, paprastai vadinamos „erkių bombomis", palengvina erkių išplitimą ir ligų perdavimą į stipresnes ir sveikesnes kolonijas. Dažniausiai naudojamos akaricidinės kontrolės priemonės turi neigiamą poveikį bičių būklei ir medaus kokybei, žmonių sveikatai ir aplinkai. Argano medis yra atogrąžų Sapotaceae šeimos atstovas. Jis yra biologiškai aktyvaus ir maistinio aliejaus šaltinis. Mes siekėme išbandyti argano medžio bioakaricidinį poveikį *Varroa destructor* erkėmis molekulinio modeliavimo metodais.

Raktažodžiai: *Varroa destructor*, *Apis mellifera*, akaricidas, *Argania*, bioiakaricidas, molekulinis modeliavimas