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THE IMPACT OF ESSENTIAL OILS OF THE LAMIACEAE FAMILY AGAINST *TYLENCHULUS SEMIPENETRANS* USING MOLECULAR MODELING METHODS

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ABSTRACT

Tylenchulus semipenetrans is an economically important plant-parasitic nematode occurring in all citrus-producing regions of the world and causing a disease called "slow decline". Chemical nematicides commonly used in agriculture have eco-toxicological effects. As a control method, attention has been paid to bio-nematicides that do not exhibit harmful effects on the ecosystem. In this study we will carry out the *in scilico* experiments in order to find the most coherent Enzyme-Ligand complex to lead to the best inhibitors of Acetyl CoA Carboxylase in Citrus *Tylenchulus semipenetrans* nematode. "Acetyl CoA Carboxylase" enzyme responsible for fatty acid synthesis in *Tylenchulus semipenetrans*, its alteration disrupting the synthesis of the surface layer, this inhibitory action is based on essential oils of aromatic plants, taking as an example the Lamiaceae family, using natural compounds extract from essential oils of *Salvia verbenaca, Lavandula stoechas, Rosmarinus officinalis,* and *Thymus ciliatus*. This study revealed for the first time that β-phellandrène from *Salvia verbenaca* gives the best docking scores compared to Biotine, the co-crystallized inhibitor of the Acetyl CoA Carboxylase, to spirotetramat as chemical insecticide already used against citrus nematode, and to the other complexes. After that, the Molecular Dynamics Simulation study showed a good result for the β-phellandrène- Acetyl CoA Carboxylase docked complex, for that we can consider that β-phellandrène extracted from *Salvia verbenaca's* essential oil as a functional inhibitor of Acetyl CoA Carboxylase activities and it can be used as good bio-nematicides against *Tylenchulus semipenetrans*.

Keywords: *Tylenchulus semipentrans*, Nematode, Molecular Docking, Bio-nematicides, Lamiaceae, Molecular Dynamics Simulation

INTRODUCTION

Citrus is grown on grafted trees worldwide in Mediterranean and subtropical climates. Numerous species of plant-parasitic nematodes have been associated with the citrus rhizosphere but few reproduce on citrus and cause damage to the trees. *Tylenchulus semipenetrans* (Fig. 1) is considered as major nematode pests, because he causes significant economic losses in multiple regions of the world (Duncan 1999). This pest is the dominant pathogenic species in most citrus regions and among diverse soil textures, information on control measures is most extensive for this nematode, whereas, a lot of studies estimate yield losses due to *Tylenchulus semipenetrans* to be in the range of 10% to 30% depending on the level of infection (Duncan and Cohn 1990).

The fight against nematodes often involves the use of chemical nematicides such as spirotetramat. The latter presents a danger to ecosystems **(**Brück, *et al*., 2009**)** new control strategies are therefore urgently needed. For this, we thought of looking for bio-nematicides to improve the production and quality of citrus fruits in the world which can be made by certain plant parts and extracts possess nematicidal properties. The application of the plant parts or extracts to nematode infested soil affects nematode directly and stimulates soil microbes that reduce nematode populations (Nandal and Bhatti 1986; Reddy *et al*., 1996; Ahmad *et al.,* 2004). Our work consists in studying the inhibition of Acetyl CoA Carboxylase, the enzyme responsible for the synthesis of fatty acids in *Tylenchulus semipenetrans*, its alteration disrupts the synthesis of the surface layer. This inhibitory action is done by ligands derived from four plants from the Lamiaceae family *Salvia verbenaca, Lavandula stoechas, Rosmarinus officinalis,* and *Thymus ciliatus*, (Fig.2) to produce a bio-nematicide.

The Lamiaceae family is very present in Algerian flora. A large part of these plants is aromatic, rich in essential oil, hence their economic and medicinal interest. The essential oils of this family are highly sought after, as they are generally endowed with interesting biological properties. The essential oils of these plants, was isolated from the aerial parts of the plant (leaves and flowers), the major component being β-phellandrène (30.3%)for *Salvia verbenaca* (Bahar *et al*., 2004), Thymol (44,2 %) in *Thymus ciliatus*. (Bousmaha *et al* 2007), α-pinèneis one of the major components in *Rosmarinus officinalis* (19.6%) (Albert *et al.,* 1996), and Fenchone (68.2%) in *Lavandula stoechas*. (Bouzouita *et al.,* 2005).In this study we will carry out the *in scilico* experiments in order to find the most coherent (Enzyme-Ligand) complex (s) to lead

to the best inhibitors of Acetyl CoA Carboxylase in Citrus *Tylenchulus semipenetrans* nematode. To do so, with the development of computer tools, molecular modeling and more precisely molecular docking quickly invested the field of biological research. The aim of molecular docking is to determine the mode of interaction of a complex composed of two or more molecules, by looking for orientations in space and favourable conformations for the binding of a ligand to a receptor.

MATERIALS AND METHODS

Molecular modeling has become a well-established research area during the last decade due to advances in computer hardware and software that have brought high-performance computing and graphics within the reach of most academic and industrial laboratories. It aims to predict the structure and reactivity of molecules. The principle of molecular modelling consists in specifying, from calculations, the position of atoms in space and calculating the energy of the generated structure.

Ligands and protein preparation

In our study we take the active ingredient of essential oils of each plant used in this experiment. The full geometrical of the ligands are downloading from PubChem Project (www.pubchem.ncbi.nlm.nih.gov) in SDF format and ligands were drawn from the software "Chemdraw" version 12.0.2.1076; (Fig.3) and saved in format "Mol", these ligands were pre-optimized by means of the Molecular Mechanics Force Field (MM+). After that, the resulted minimized structures were further refined using the semi-empirical AM1 (Stewart, J.J.P., 2007) with the Polak-Ribiere conjugate gradient algorithm of 0.01 kcal/(Åmol). All methods are implemented in "Hyperchem" "version 8.0.10 and saved in "Mol" format, for molecular docking with the Molecular Operating Environment" MOE "software 2013.

After that, we downloaded Acetyl CoA Carboxylase enzyme with 1BDO code from "RCSB" or "PDB" (Protein Data Bank) (Fig.4) with a resolution of 1.80 A. This is selected for modeling studies; the energy of the proteins structures is minimized using the Energy minimization algorithm of MOE tool. These energies of proteins are calculated by MOE using Amber10: EHT force field with conjugant gradient method.

Molecular Docking Protocol

Docking calculations were carried out using standard default parameter settings in the MOE software package (Molecular Operating Environment (MOE, 2013). In this molecular docking program, the proteins are considered as a rigid structure and the ligands are considered as flexible compounds (Abdelli *et al* 2020).

In general, the protein structure with a resolution between 1.5 and 2.5 Å have a good quality for further studies (Clément & Slenzka, 2006; Didierjean & Tête-Favier, 2016), whereas, the resolution value of 1BDO is 1.8 Å. Molecular Docking is the step of the selection which consists of placing the ligand in the active site of the protein and sampling the positions, conformations and orientation (poses) possible to have the most stable complex. After that**,** the ranking step, which consists in evaluating the affinity between the ligand and the protein and giving a score to poses obtained during the Docking phase. This score helps us to remember the best pose among all that proposed (Boucherit 2012).

Molecular dynamics simulation

The molecular dynamics simulation study was carried out for the ligand that was declared as the best among the selected molecules. The molecular dynamics simulation study was performed by iMODS. It is a fast, user-friendly and effective molecular dynamics simulation tool that can be used efficiently to investigate the structural dynamics of the protein complexes. It provides the values of deformability, B-factor (mobility profiles), eigenvalues, variance, co-variance map and elastic network. For a complex or protein, the deformability depends on the ability to deform at each of its amino acid residues. The eigenvalue has relation with the energy that is required to deform the given structure and the lower the eigenvalue, the easier the deformability of the complex. Moreover, the eigenvalue also represents the motion stiffness of the protein complex. IMODS is a fast and easy server for determining and measuring the protein flexibility (Awan *et al.,* 2017; Prabhakar *et al.,* 2016; López-Blanco *et al.,* 2014).

RESULTS AND DISCUSSION

Molecular docking simulation

The best conformations of the ligands were analyzed for their binding interactions and were evaluated by the binding free energies and bonds interactions between ligand atom and active site residues. The results calculations docking details received after dock all ligands with Acetyl CoA Carboxylase 1BDO target are listed in the Table 1

The molecules that had the lowest binding energy of docking score were considered the best molecule and inhibiting the target receptor as the lower binding energy corresponds to higher binding affinity (Simon 2016).
The 1BDO-ligands complexes formed are shown in (Fig 5-10)

The best score obtained after docking is that of spirotetramata chemical nematicides with a score of -4.9816 kcal mol, followed by Biotine the reference ligand with a score of -4.6061 kcal mol, the values of

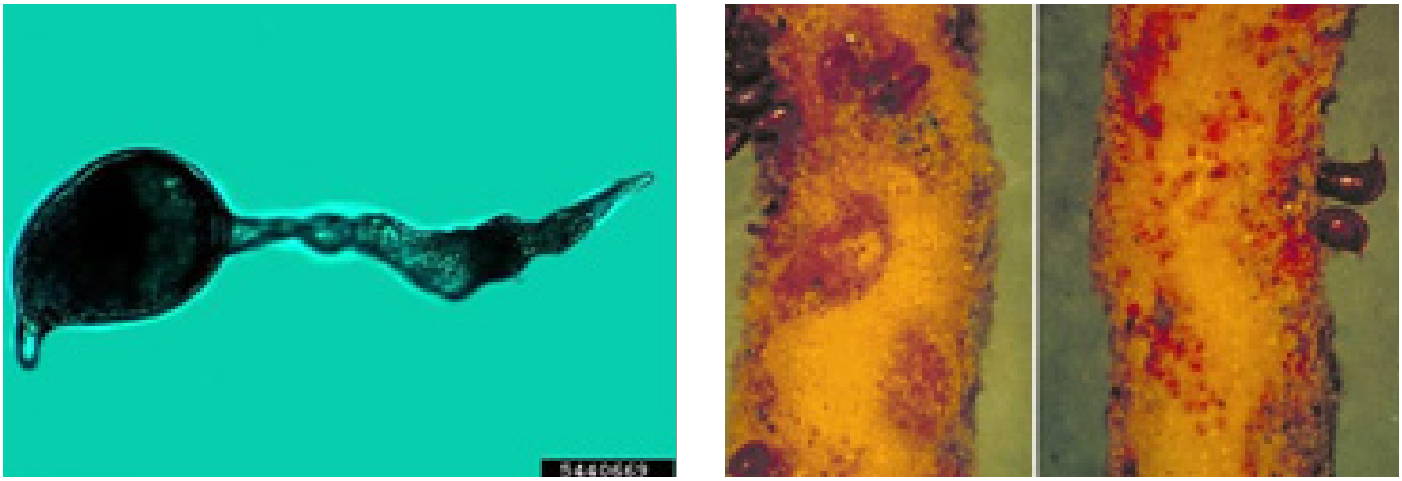
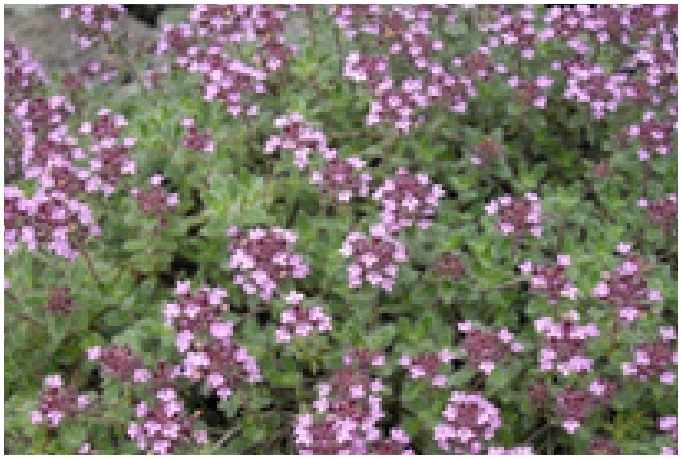


Fig. 1.*Tylenchulus semipenetrans* citrus nematode and his site feeding

Salvia verbenaca



Thymus ciliatus

Lavandula stoechas

Rosmarinus officinalis

Fig.2 All plants used in our study from the Lamiaceae family.

the other scores(-4.1921 Kcal mol) ofβ-phellandrène, (-4.0341) for Fenchone, (-4.0278 kcal mol) of Thymol, and (-3.2004) for α-pinène.We compared all the values score complexes; we found that complex formed by 1BDO-Spirotetramat has the lowest value energy and giving the best docking score compared to Biotine co-crystallized inhibitor, on the other hand, β-phellandrène gives almost the same values of the score (-4.1921 Kcal/mol and -4.9816 Kcal/mol respectively) therefore we can consider that the two complexes 1BDO-β-phellandrène and 1BDO-Spirotetramat are stables with higher binding affinity. According to these docking results we can classify β-phellandrène as the good natural inhibitor of the enzyme 1BDO, compared to the chemical pesticide

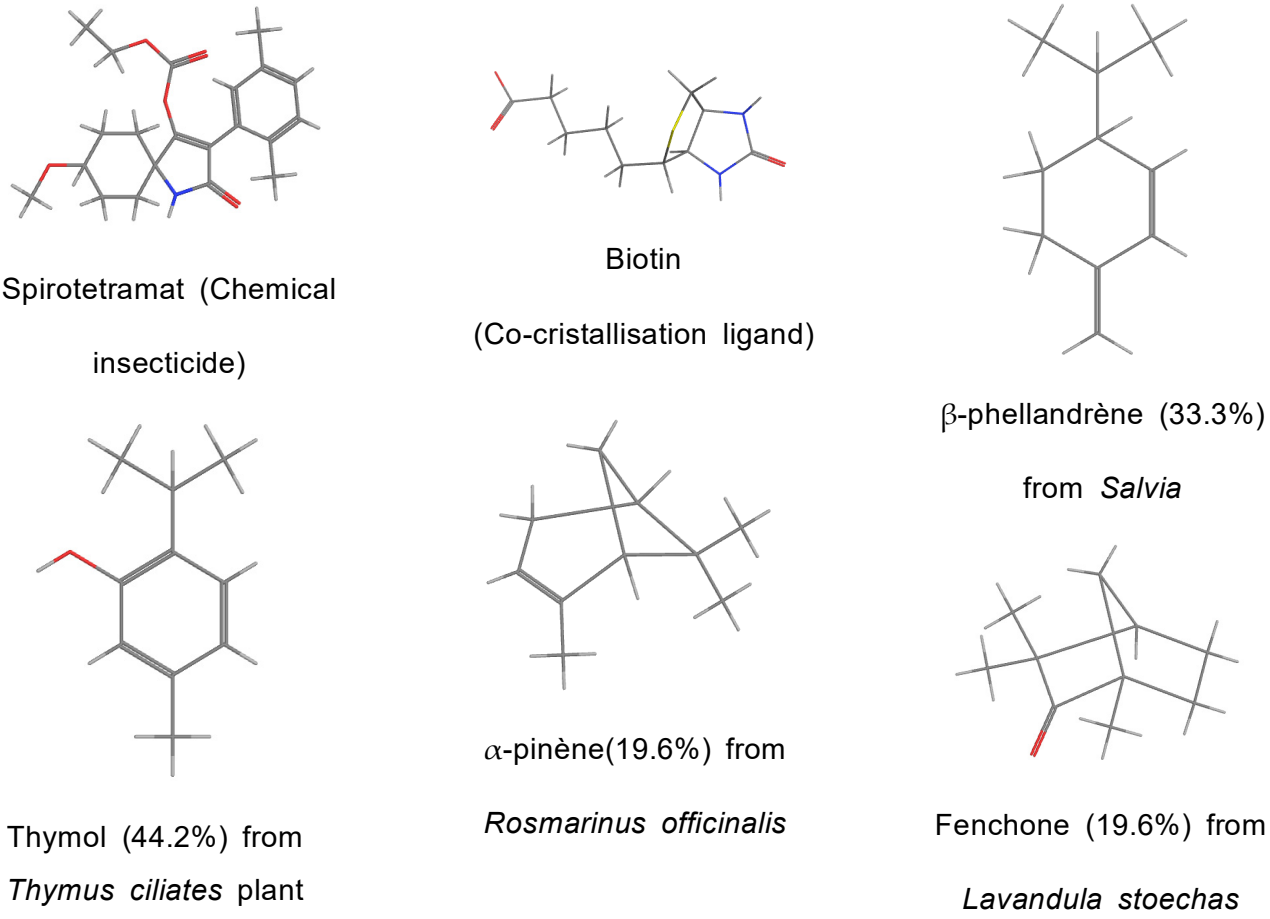


Fig. 3. 3D representations of the all the ligands used in the experiment

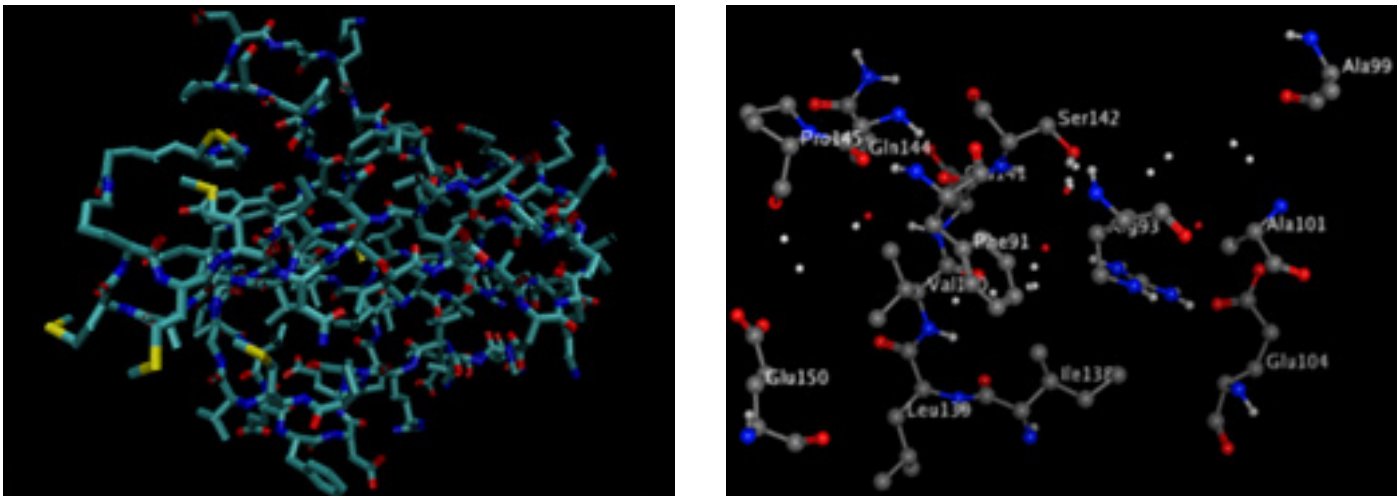


Fig.4. 1BDO enzyme 3D structure

"Spirotetramat", and to all ligands studied.

Molecular dynamics simulation

The Molecular Dynamics Simulation results are showed in the Fig.11.

Fig.11.A illustrates the normal mode analysis (NMA) of 1BDO-β-phellandrène complex. The deformability graph of the complex illustrates the peak in the graphs correspond to the regions in the protein with deformability Fig.11. B. The B-factor graph of the complex gives easy visualization and understanding of the comparison between the NMA and the PDB field of the complex Fig.11. C. The eigenvalue of the complex is illustrated in Fig.11. D. The docked complex generated eigenvalue of 7.385787e-04. The variance graph indicates the individual variance by red colored bars and cumulative variance by green colored bars Fig.11. E.Fig.11. F. illustrates the co-variance map of the complexes where the correlated motion between a pair of residues is indicated by red color, uncorrelated motion is indicated by white color and anti-correlated motion is indicated by blue color. The elastic map of the complex shows the connection between the atoms and darker gray regions indicate stiffer regions Fig.11. G.

Table 1. The docking results (binding energy) of all ligands and the controls along their respective number of hydrogen bonds as well as interacting amino acids

Names of ligands	SP Docking Score (Binding Energy) (Kcal/mol)	Interacting residues of the target	Types of bonds	Distance (Å)	Energies (kcal-mol)
Spirotetramat	-4.9816	/	/	/	/
Biotine	-4.6061	O -ILE 138 N -SER 142 NH1-ARG 93 N -SER 142 NH1-ARG 93	H-donor H-accepteur H-accepteur H-accepteur ionic	2.97 2.95 3.05 3.32 3.05	-3.8 -4.7 -4.7 -1.2 -4.1
β-phellandrène	-4.1921	/	/	/	/
Fenchone	-4.0341	/	/	/	/
Thymol	-4.0278	O1- VAL 140 6-ring- GLU 141	H-donor pi-H	2.86 3.78	-1.9 -0.8
α-pinène	-3.2004	/	/	/	/

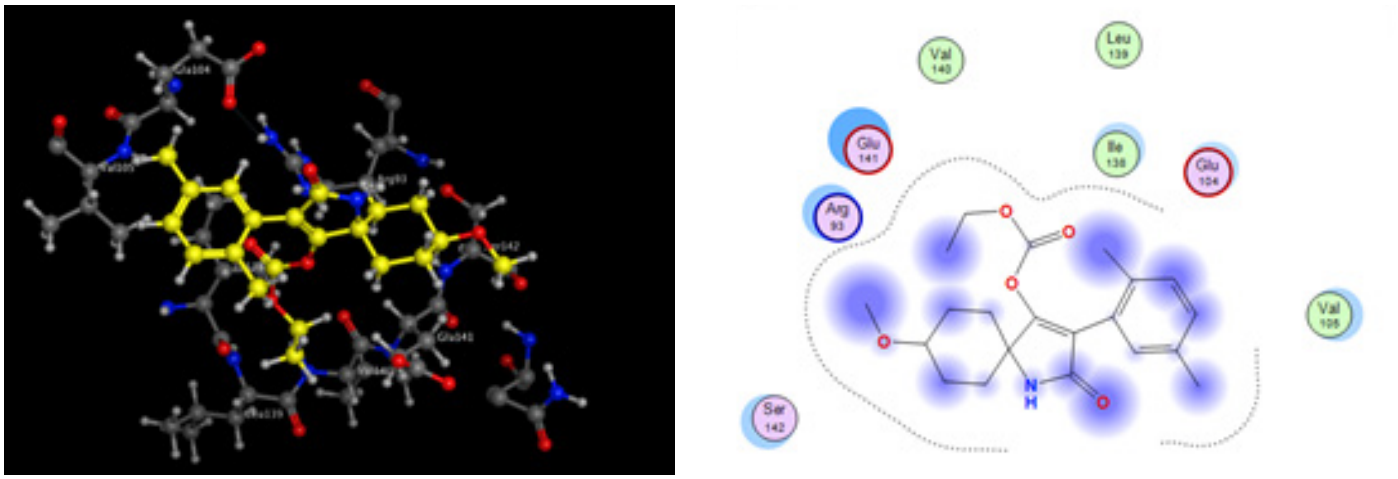


Fig.5. 1BDO-Spirotetramat complex

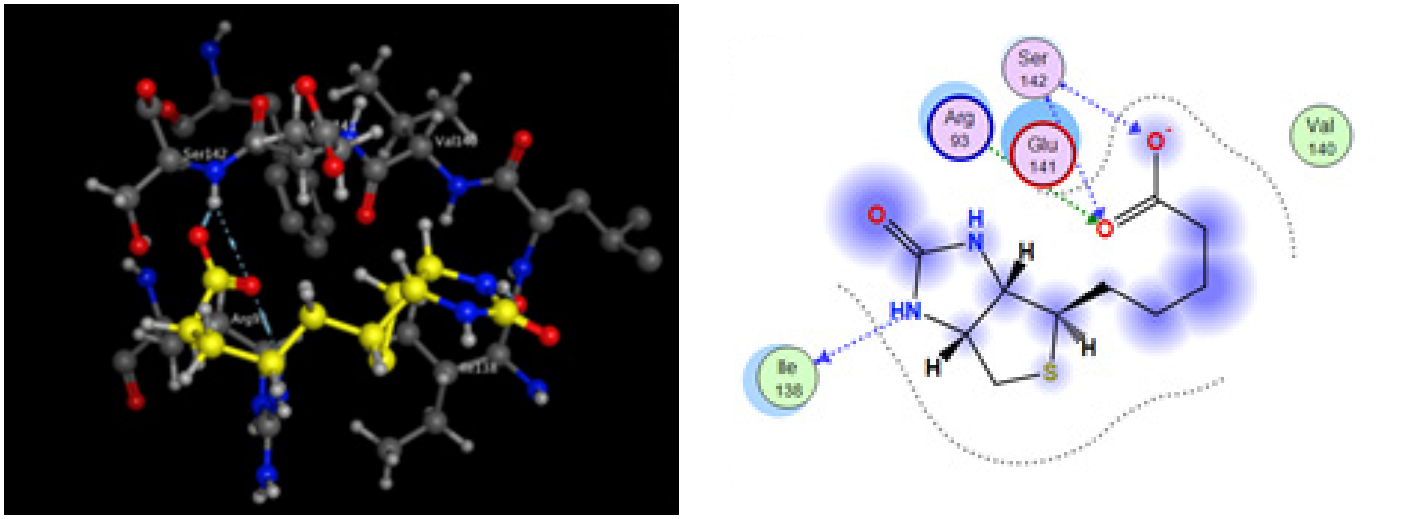


Fig.6.1BDO-Biotine complex

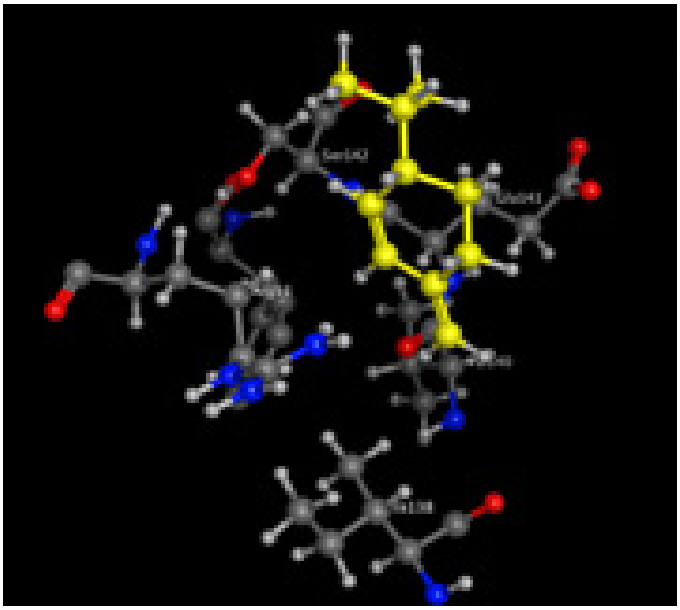
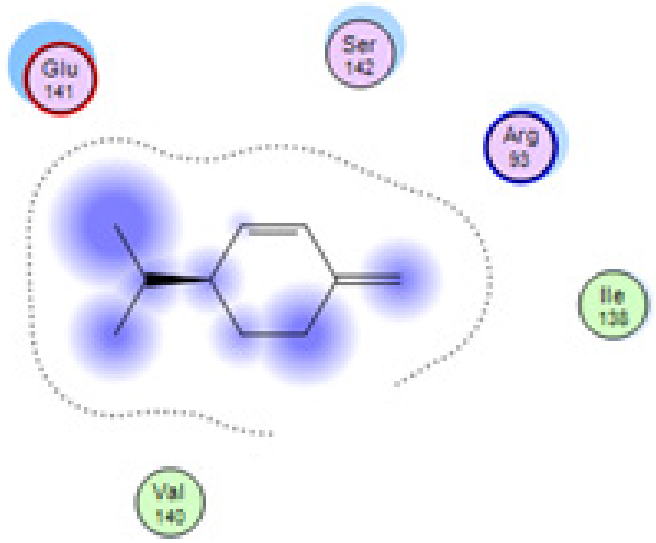



Fig.7.1BDO-β-phellandrène complex

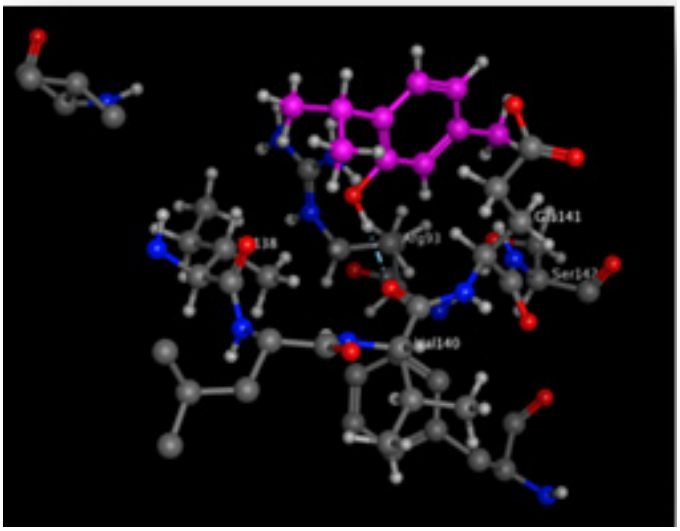
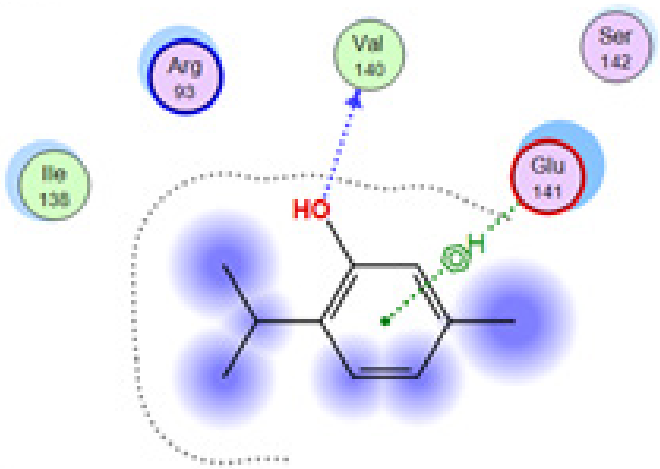



Fig.8.1BDO-Thymol complex

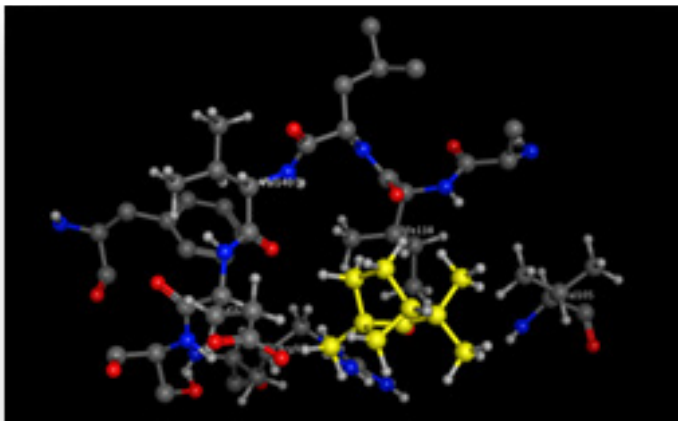
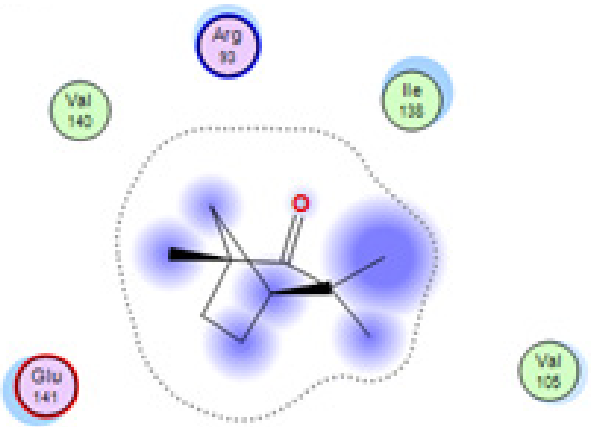
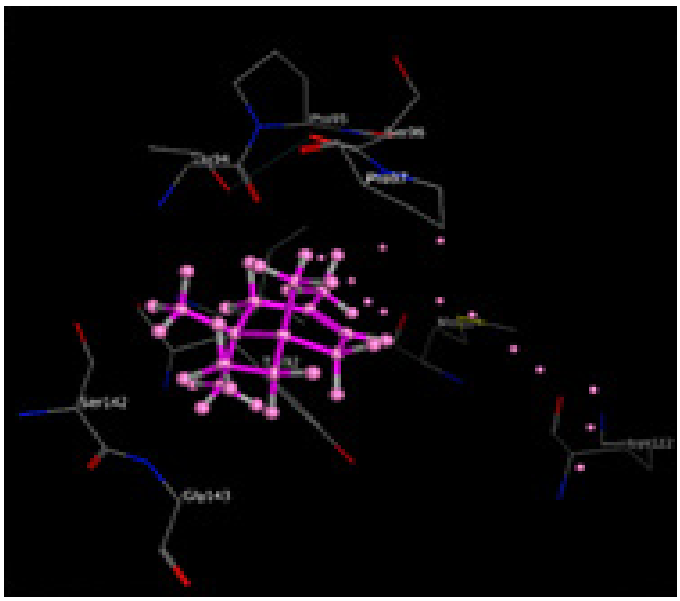



Fig.9.1BDO- Fenchone complex

From the molecular dynamics study of 1BDO-β-phellandrène docked complex, it is clear that the complex had a very good amount of deformability Fig.11.B as well as it had high eigenvalue of 7.385787e-04, for this reason, the deformability would be quite difficult for the complex Fig.11.D and also represents the motion stiffness of the protein complex.

However, the variance map showed high degree of cumulative variances than individual variances Fig.11. E. The co-variance and elastic network map also produced satisfactory results Fig.11.F and Fig.11. G.



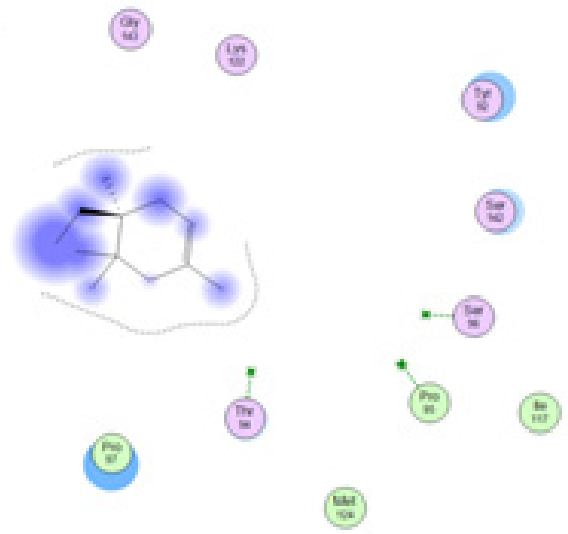
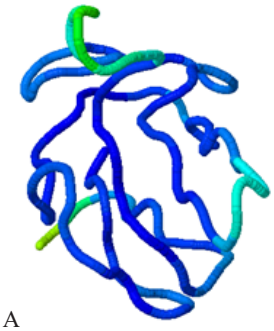
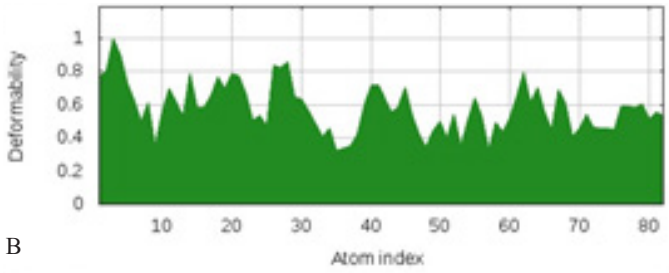


Fig.10.1BDO- α-pinène complex



A



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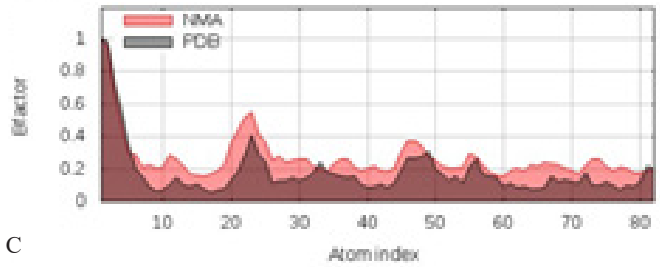

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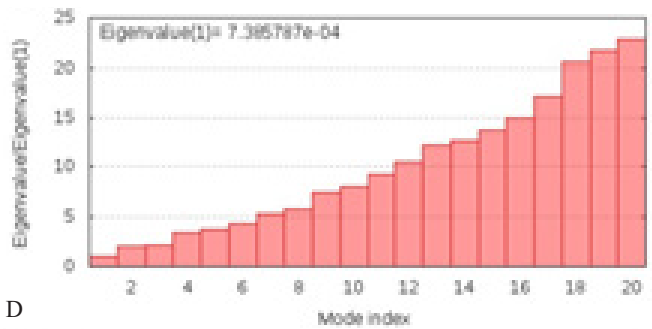

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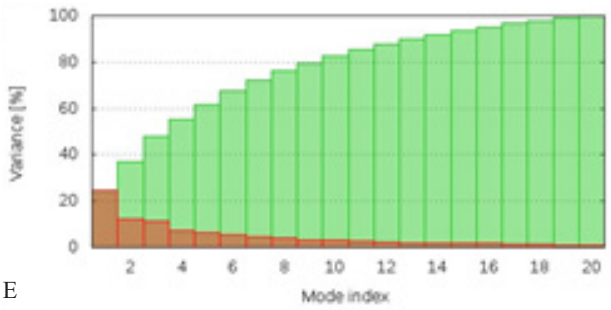

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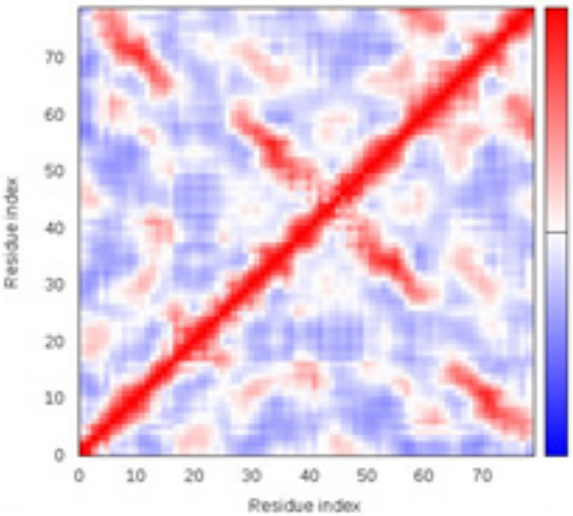

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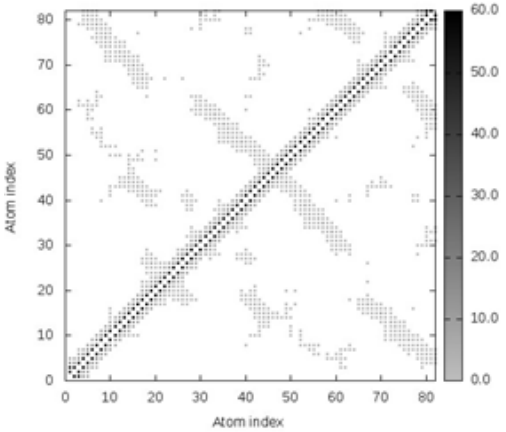

G

Fig.11. Results of molecular dynamics simulation of 1BDO-β-phellandrène Receptor. (A) NMA mobility, (B) deformability, (C) B-factor, (D) eigenvalues, (E) variance (red color indicates individual variances and green color indicates cumulative variances), (F) co-variance map (correlated (red), uncorrelated (white) or anti-correlated (blue) motions) and (G) elastic network (darker gray regions indicate more stiffer regions) of the complex.

CONCLUSION

The chemical nematicides like spirotetramat commonly used in agriculture have eco-toxicological effects. As control means, we have been interested in bio-nematicides that do not have harmful effects on the ecosystem. For this purpose, we based in the present study on aromatic plants essential oils, taking as an example the Lamiaceae family**.** The aim of our study is to investigate the potent of natural's compounds extracted from Lamiaceae family plants such asβ-phellandrène, Fenchone, Thymol, and α-pinèneas inhibitors for Acetyl CoA Carboxylase target, the enzyme responsible for the synthesis of fatty acids in *Tylenchulus semipenetrans*, its alteration disrupts the synthesis of the surface layer. These compounds are tested *in silico* study for the inhibition to this enzyme. Molecular docking used to study interaction between new compounds "β-phellandrène" and 1BDO with score energy investigation and Molecular dynamics simulation have been performed to verify *in silico* the properties of this best ligand. The top ligand β-phellandrène which is the major component (30.3 %), in *Salvia verbenaca* has high binding affinity (Score) and one almost stable interaction with 1BDO target.

Finally, we can suggest from this *in silico* study that *Salvia verbenaca* essential oils components can blocks the receptorofAcetyl CoA Carboxylase and could present a power asa bio-nematicide compared to spirotetramat, a chemical pesticide used against the citrus nematode *Tylenchulus semipenetrans.*

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