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IMPORTANCE OF ESSENTIAL OILS FOR THE BIOCONTROL OF *TYLENCHULUS SEMIPENETRANS* NEMATODE POPULATIONS THROUGH MOLECULAR MODELING METHODS

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Abstract

In Algeria, the number of citrus trees ranks second after olive trees, but their economic importance clearly places them at the top of fruit production. The nematode *Tylenchulus semipenetrans* attaches to citrus roots and causes slow citrus dieback. The chemical nematicides used in agriculture have eco-toxicological effects, whereas bio-nematicides do not harm the ecosystem. In this context, the present study focuses on essential oils from aromatic plants, specifically from the Lamiaceae family, using two species: *Thymus algeriensis* and *Rosmarinus officinalis*. Some ligands derived from these essential oils have an inhibitory effect on Acetyl-CoA Carboxylase, an enzyme responsible for the biosynthesis of fatty acids in *Tylenchulus semipenetrans*. Using molecular modeling methods, including molecular docking with the Molecular Operating Environment (MOE) software, the study reveals for the first time that linalool, a major component of *Thymus algeriensis*, is a functional inhibitor of Acetyl-CoA Carboxylase activities and can be used as an effective bio-insecticide against *Tylenchulus semipenetrans* to protect citrus trees.

Introduction

Algeria enjoys favorable conditions for the development of citrus due to Mediterranean climate and geomorphological characteristics (Hassani 2006). The overall production of citrus has been estimated at more than 14 million of quintals in 2018 (Bouazghi 2019).

Agriculture is often threatened by phytophagous insects and nematodes. Chemical control is a popular method of protecting agricultural yields, but it is likely to be harmful to humans and the environment. This results in fruit toxicity as well as the destruction of beneficial fauna, highlighting the need to use specific attractants (Ros 1997).

The use of aromatic plants has a broad spectrum. The technique consists in extracting ligands from essential oils of these aromatic plants to fight against the different pests of crops. The advantages of this method consist in an absolute specificity of absence of toxicity and in an important efficiency in the frame of the protection of cultures.

However, the rate of production could have been better with the use of bio-nematicides to improve the quantity and quality of citrus fruits, since 10 to 30% of crop loss is caused by *Tylenchulus semipenetrans*, a major parasite of Citrus Fig.1. This nematode is widely distributed in the world (Duncan 2005, Sorribas 2008, Verdejo-Lucas *et al*. 1992) and in the Mediterranean (Verdejo-Lucas 1992, Inserra 1994, Duncan *et al.* 1992).

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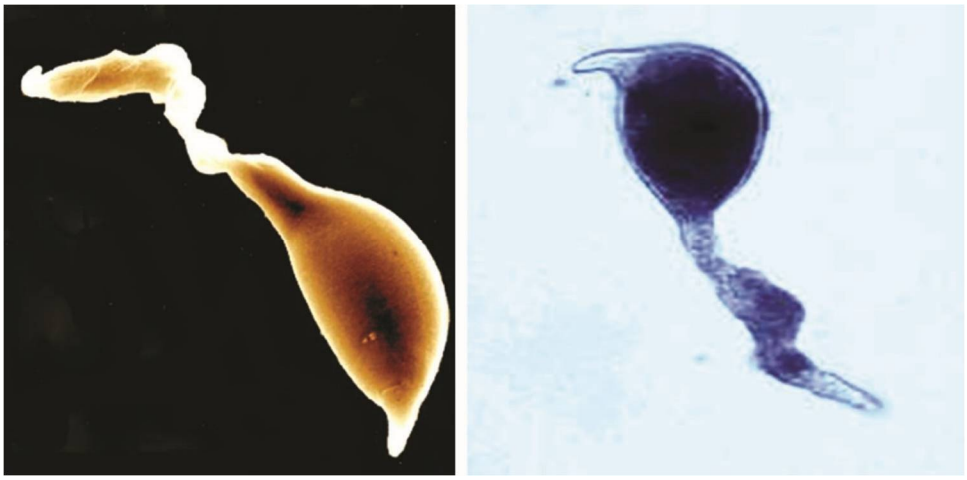

Fig. 1. *Tylenchulus semipenetrans* citrus nematode.

It is an obligate parasite which means that it needs living plant tissues (roots) in order to live and reproduce. It is responsible for the slow decline of citrus fruit Fig. 2 (El-Borai *et al* .2002).

Algeria, by its geographical situation, offers a rich and diverse vegetation. A large number of aromatic plants such as Lamiaceae grow there spontaneously. Interest in these plants has continued to grow in recent years (Kholkhal 2014). Because of their abundance in nature and their properties to be used in agriculture as bio-insecticides thanks to their essential oils.

Fig. 2. Slow decline of citrus fruit caused by *Tylenchulus semipenetrans.*

To do so, with the development of computer tools, molecular modeling and more precisely molecular docking quickly invested the field of biological research. The main purpose of molecular docking is to predict the most favorable conformation (position and relative orientation) of the ligand within its receptor.

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 two plants from the Lamiaceae family *Thymus algeriensis* and *Rosmarinus officinalis*, Fig. 3 to produce a bio-nematicide.

Fig. 3. *Thymus algeriensis and Rosmarinus officinalis.*

The purpose of the study is to 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.

Materials and Methods

Ligand and protein preparation: The ligands used in this work are chosen according to the most important yield in each part of these two plants (leaf and fruit), Linalool was by far the major component (43,3 %) of *Thymus algeriensis* (Dob *et al*. 1994**)**, and camphor is the major component in *Rosmarinus officinalis* (39,27%) (Tahri 2013). The chemical structure of these ligands was obtained from the database “Pubchem” (Table 1), and ligands were drawn from the software “Chemdraw” version 12.0.2.1076; and saved in format “Mol”, and then optimized the ligands with the software “Hyperchem” "version 8.0.10 and saved in “Mol” format, for molecular docking with the Molecular Operating Environment" MOE "software version 2013 .

Downloaded Acetyl CoA Carboxylase enzyme with 1BDO code from “RCSB” or “PDB” (Protein Data Bank) with a resolution of 1.80A°. In general, the protein structure with a resolution between 1.5 and 2.5 Å have a good quality for further studies (Clément and Slenzka 2006), whereas, the resolution value of 1BDO “1.80A°” belongs to this interval.

The method is based on molecular modeling. Docking basically consists of two steps:

- 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.
- Scoring is 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).

Docking calculations were carried out using standard default parameter settings in the MOE software package (Molecular Operating Environment). In this molecular docking program, the flexibility of ligands is considered while the proteins are considered as a rigid structure. At the end of molecular docking, the best conformations of the ligands were analyzed for their binding interactions and were evaluated by the binding free energies (S-score, kcal/mol) and bonds interactions between ligand atom and active site residues.

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-varience 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 (López-Blanco *et al.* 2014, Prabhakar *et al.* 2016, Awan *et al.* 2017).

Results and discussion

The results calculations docking details received after dock all ligands with Acetyl CoA Carboxylase target are listed in the Table 1.

Table 1. Docking results of ligands and the controls along their respective number of hydrogen bonds as well as interacting amino acids.

Names of ligands	SP docking score (Kcal/mol)	Interacting residues	Types of bonds	Distance (Å)	Energies (kcal-mol)
Spirotetramat	-4.9816	No interactions	No interactions	No interactions	No interactions
Biotine	-4.6061	O -ILE 138	H-donor	2.97	-3.8
		N -SER 142	H-accepteur	2.95	-4.7
		NH1-ARG 93	H-accepteur	3.05	-4.7
		N -SER 142	H-accepteur	3.32	-1.2
		NH1-ARG 93	ionic	3.05	-4.1
Linalool	-4.3169	N - SER 142	H-accepteur	3.07	-0.6
Camphor	-3.5505	No interactions	No interactions	No interactions	No interactions

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 *et al* 2017).

The 1BDO-ligands complexes formed are shown in Figs 5-8.

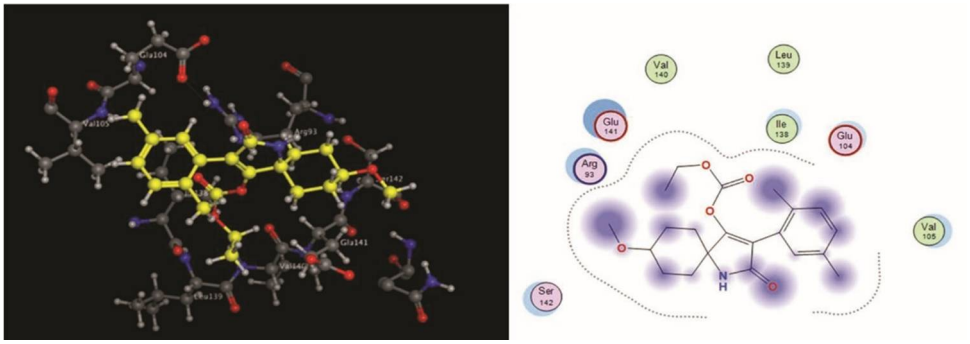

Fig. 5. 1BDO-Spirotetramat complex.

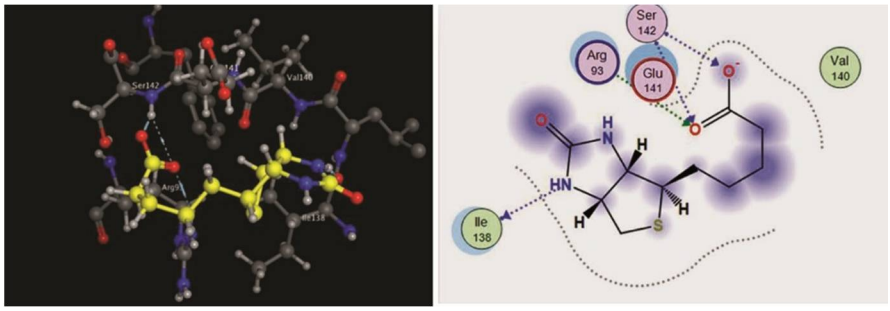

Fig. 6 .1BDO-Biotine complex.

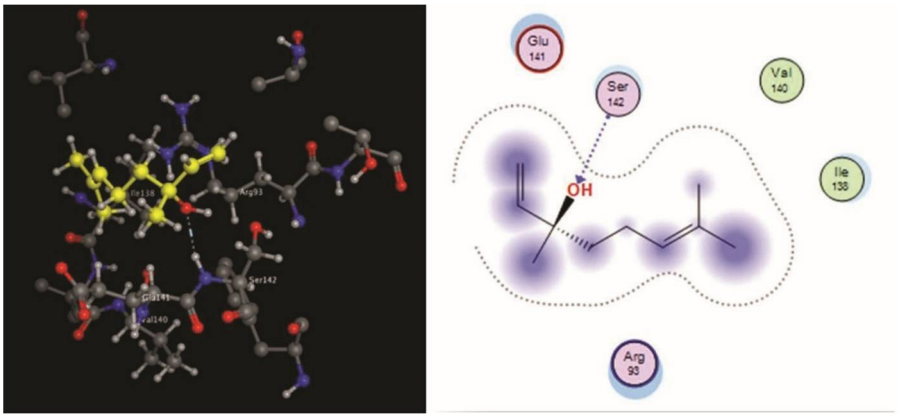

Fig. 7. 1BDO-Linalool complex.

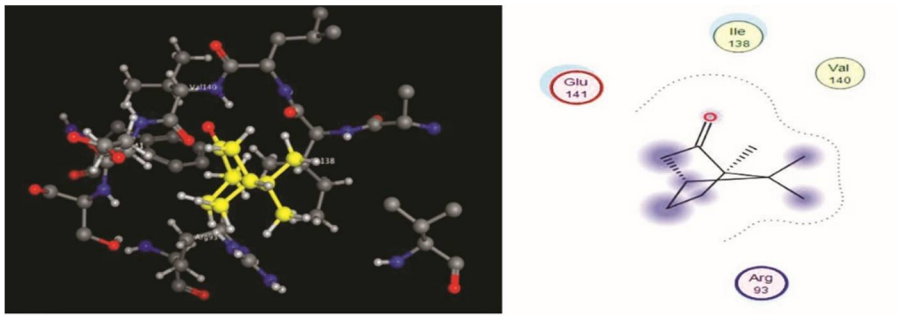

Fig. 8. 1BDO- Camphor complex.

The best score obtained after docking is that of Spirotetramat, with a score of -4.9816 kcal/mol, followed by Biotine, the reference ligand, with a score of -4.6061 kcal/mol. Other scores include -4.3169 kcal/mol for Linalool and -3.5505 kcal/mol for Camphor. All the score values for the complexes were compared, and it was found that the complex formed by 1BDO - Spirrotetramat has the lowest energy value, giving the best docking score compared to the Biotine co-crystallized inhibitor. On the other hand, Linalool has one hydrogen interaction with SER 142, while the 1BDO-Spirrotetramat complex has no hydrogen interactions. Despite this, both complexes yield almost identical scores (-4.3169 kcal/mol and -4.9816 kcal/mol, respectively),

indicating that the two complexes, 1BDO -Linalool and 1BDO -Spirrotetramat, are stable and have higher binding affinities. Based on these docking results, Linalool can be classified as a good inhibitor of the enzyme 1BDO when compared to all the ligands studied.

The Molecular Dynamics Simulation results are showed in the Fig 9 .

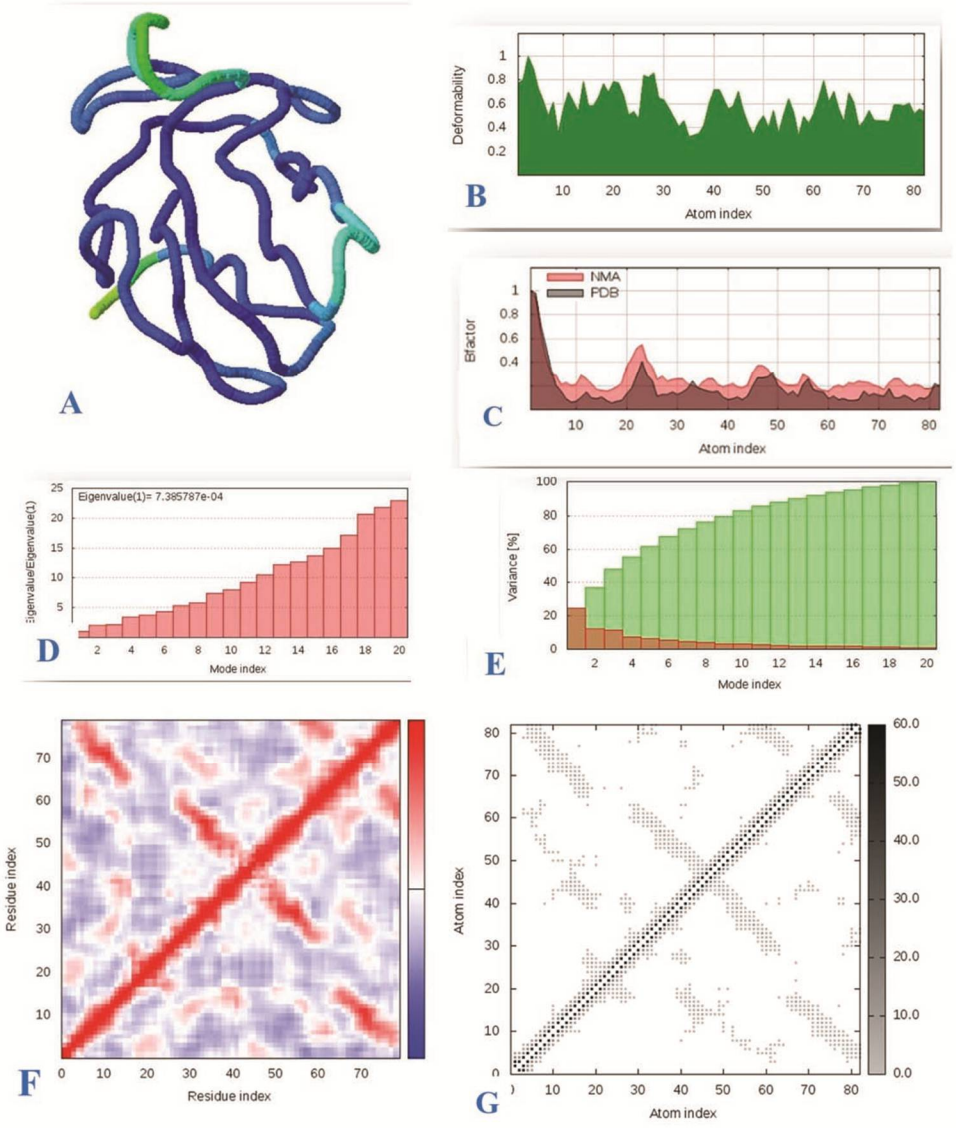

Fig. 9. Results of molecular dynamics simulation of 1BDO - Linalool Receptor. (a) NMA mobility, (b) deformability, (c) B-factor, (d) eigenvalues, (e) variance (red color indicates individual variances and green color indicates cumulative variances), (f) co-variance map (correlated (red), uncorrelated (white) or anti-correlated (blue) motions) and (g) elastic network (darker gray regions indicate more stiffer regions) of the complex.

Fig. 9A illustrates the normal mode analysis (NMA) of 1BDO - Linalool complex. The deformability graph of the complex illustrates the peak in the graphs correspond to the regions in the protein with deformability (Fig. 9 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. 9C). The eigenvalue of the complex is illustrated in (Fig. 9D) . 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. 9E). (Fig. 9 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. 9G) .

From the molecular dynamics study of 1BDO- Linalool docked complex, it is clear that the complex had a very good amount of deformability (Fig. 9B) as well as it had high eigenvalue of 7.385787e-04, for this reason, the deformability would be quite difficult for the complex (Fig. 9D) and also represents the motion stiffness of the protein complex.

However, the variance map showed high degree of cumulative variances than individual variances (Fig. 9E). The co-variance and elastic network map also produced satisfactory results (Fig. 9F) and (9G) .

This present research interested to the molecular interactions of Acetyl CoA Carboxylase enzyme responsible for the biosynthesis of fatty acids of *Tylenchulus semipenetrans*, and inhibitory ligands resulting from the extraction of essential oils from Lamiaceae family, using molecular docking which is one of the methods of molecular modeling. The results obtained using the software "MOE" reveal that the complex (1BDO-Linalool) with the lowest score, resembles that of Spirotetramat with a small difference. Nevertheless, the latter is a chemical insecticide against Acetyl CoA Carboxylase. It is considered that *Thymus algeriensis* is a good bio-nematicide against *Tylenchulus semipenetrans*.

This study revealed that Linalool is a functional inhibitor of Acetyl CoA Carboxylase activities, and it can be concluded that the essential oil components of *Thymus algeriensis* (Lamiaceae family) present strong potential as a bio-insecticide against *Tylenchulus semipenetrans*.

References

Awan F, Obaid A, Ikram A and Janjua H 2017. Mutation-structure-function relationship based integrated strategy reveals the potential impact of deleterious missense mutations in autophagy related proteins on hepatocellular carcinoma (HCC): A comprehensive informatics approach. Int. J. Mol. Sci. **18**(1): 139.

Bouazghi A 2019. Production d'agrumes en Algérie. Ministère de l'agriculture, du développement rural et de la pêche. Article du journal El Moudjahed.

Boucherit H 2012. Etude théorique des interactions intervenant dans l'inhibition de la méthionine aminopeptidase de *Mycobacterium tuberculosis* par diverses molécules. Thèse de magister en biochimie. Constantine: Université Mentouri Constantine. Algérie. pp. 71.

Brück E, Elbert A, Fischer R, Krueger S, Kühnhold J and Klueken AM 2009. Movento (r), un insecticide ambimobile innovant pour le contrôle des insectes nuisibles en agriculture: Profil biologique et performance sur le terrain. Prot. Crop. **10**: 838-844.

Clément G , and Slenzka K 2006. Fundamentals of space biology: Research on cells, animals, and plants in space. Springer Sci. Business Media. pp. 18.

Dob T, Darhmane D, Benabdelkader T and Chelghoum TC 2006. Studies on the essential oils and antimicrobial activity of *Thymus algeriensis* Boiss. & Reut. Int. J. Aromatherapy **16**(2): 95-100.

Duncan LW 2005. Nematode parasites of citrus. In: Plant parasitic nematodes in subtropical and tropical agriculture (eds. M. Luc, RA Sikora, and J. Bridge). 2nd ed. CABI Publishing, Wallingford, UK. pp. 593-607.

El-Borai FE, Duncan LW and Graham JH 2002. Infection of citrus roots by *Tylenchulus semipenetrans* reduces root infection by *Phytophthora nicotianae*. J. Nematol. **34**: 384-389.

Hassani I 2006. Laboratoire de biotechnologies végétales, équipe plante du sud. Faculté des Sciences d'Agadir, Maroc.

Inserra RN, Duncan LW, O'Bannon H and Fuller SA 1994. Citrus nematode biotypes and resistance of citrus rootstocks in Florida, Nematol. Circ. 205: 4.

Kholkhal F 2014. Etude phytochimique et activité antioxydante des extraits des composés phénoliques de *Thymus ciliatus* ssp. *coloratus* et ssp. *euciliatus*. Thèse. Université de Tlemcen.

López-Blanco JR, Aliaga JI, Quintana-Ortí ES and Chacón P 2014. iMODS: Internal coordinates normal mode analysis server. Nucleic Acids Res. **42**(W1): W271-276.

Prabhakar PK, Srivastava A, Rao KK and Balaji PV 2016. Monomerization alters the dynamics of the lid region in *Campylobacter jejuni* CstII: An MD simulation study. J. Biomol. Struct. Dyn. **34**(4): 778-791.

Ros JP 1997. A general view of recent advances of attractants and traps against *Ceratitis capitata* (Wied) (Dipt.: Teph.). Proc. (IOBC/WPRS) Bull. **20**(8): 137-144.

Sorribas FJ, Verdejo-Lucas S, Pastor J, Ornat C, Pons J and Valero J 2008. Population densities of *Tylenchulus semipenetrans* related to physicochemical properties of soil and yield of Clementine mandarin in Spain. Plant Dis. **92**: 445-450.

Simon L, Imane A, Srinivasan K. Pathak KL and Daoud I 2017. In silico drug-designing studies on flavanoids as anticolon cancer agents: Pharmacophore mapping, molecular docking, and Monte Carlo method-based QSAR Modeling. Interdisciplinary Sciences: Computational Life Sciences 9(3): 445-458.

Tahri M 2013. Les composés organiques et minéraux des aromatiques médicinaux *Rosmarinus tournefortii* et *Rosmarinus officinalis*, qui poussent dans l'est du Maroc. Université Mohammed Premier.

Verdejo-Lucas S 1992. Seasonal population fluctuations of *Meloidogyne* spp. and the *Pasteuria penetrans* group in kiwi orchards. Plant Dis. **76**: 1275-1279.

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