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Full Length Research Paper

Study of the antifungal activity of essential oil extracted from seeds of *Foeniculum vulgare* Mill. for its use as food conservative

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The objective of this study was to evaluate *in vitro* antifungal activity of dry seeds' essential oil extracted from *Foeniculum vulgare* Mill. The screening of antifungal activity was carried out by the diffusion and microatmospheric method. Results obtained indicate that fennel seed essential oil has an inhibiting effect on the tested strains. The method of dilution enabled us to evaluate the values of the minimum fungistatic concentration (MFSC) and the minimum fungicidal concentration (MFCC). These concentrations lie between 625 and 1250 µg.ml^{-1}. The antifungal index (AI_{50}) was also estimated, *Alternaria* strain seems to be most sensitive with an AI_{50} close to 26.22 ± 0.693 µg.ml^{-1}. With the rise of this study, it is shown that fennel seed essential oil could be regarded as a very promising preservative for food industry which is able to prevent the mycelia growth responsible of food deterioration.

Key words: Essential oil, antifungal activity, *Foeniculum vulgare* Mill.

INTRODUCTION

The microbiological quality of a food constitutes is one of the essential bases of its aptitude to satisfy the safety of the consumer. A food, exposed to deterioration by the fungus can have a decreasing in its sensory, nutritive and medical characteristics (Rozier et al., 1986). In spite of improvements in food conservation techniques, nature of food conservatives is an important question for the public health (Burt, 2004). Substantial quantities of stored food products are attacked by bacteria and fungus around the world. In particular, in developing countries, stored food suffers serious damage, driving with economic losses and health hazard. Fungus are also responsible for the formation of taste and the production of made up and allergenic mycotoxins (Ownagh et al., 2010). To face the problems of contamination of foodstuffs, the rise of chemistry allowed the appearance and the application of new chemical substances as synthetic food conservatives (Moll, 1998). The latter was usually employed to prevent the deterioration of food (Nakahara et al., 2003). Thereafter, several synthetic conservatives are limited in several countries, because of their undesirable toxicological effects in the long run, including the cancerogenicity (Chahardehi et al., 2010). In the same way, consumers seeking a more natural food encouraged the research, the development and the application of new natural products having antimicrobial activities with an aim of using them as alternatives to the synthetic conservatives in food industries.

Aromatic plants are traditionally employed for seasoning and prolongation of shelf life of food (Wang, and Huang, 2010). The majority of their properties are due to the essential oils produced by their secondary metabolism (Rashid et al., 2010). Great importance is given to these oils by the industry and scientific research for two reasons: on the one hand, their antifungal activity (Dung et al., 2008), on the other hand, the majority of essential oils are classified in the list of the substances GRAS, which make them useful as natural preservatives in food industries (Gachkar et al., 2007; Rasooli et al., 2008). Among the aromatic plants, we consider the fennel, in which its seeds have several uses (culinary, pharmaceutical, etc). Singh et al. (2006) reported that the *trans*-anethole is the component responsible of the

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antifungal activity. A research in scientific literature indicates that there are few reports of studies on the antimicrobial properties of seeds' essential oil extracted from fennel. In this context, this study is undertaken to evaluate the antifungal activity of essential oil of fennel seeds in order to propose as a conservative in the food industry.

MATERIALS AND METHODS

Plant materials

The material or the vegetable body selected in the present study is represented by dry seeds of bulbous fennel (*F. vulgare* Mill.). These latter were bought, in dried form, at an arborist. They originate from Ain Ouelman, wilaya of Setif, Algeria.

Oil extraction

The extraction of fennel seed essential oil (EO) was made by a hydrodistillation using the Clevenger type apparatus (Clevenger, 1928). 100 g of fennel seed was subjected to hydrodistillation for 3 h and the oils obtained were dried over anhydrous sodium sulfate (Özcan and Chalchat, 2006).

Origin and choice of fungus strains

The fungus strains were selected for their implication in the contamination and the deterioration of the foodstuffs and production of mycotoxins. *Alternaria*, *Aureobasidium, Aspergillus fumigatus* CIP 1082.74, *Fusarium, Penicillium, Rhizopus* and *Trichophyton rubrum* CIP 2043.92 were provided by the Genius Microbiological and Applications Laboratory of the Nature Science Faculty, University Mentouri, Constantine.

Preparation of spore suspension

The fungi were grown on potato-dextrose agar (PDA) plates at 30°C for 2 to 7 days, after which, spores were harvested from sporulating colonies and suspended in sterile distilled water. The concentrations of spores in suspension were adjusted to 10^6 spores/ml.

Screening of antifungal activity

The disc diffusion method was used for antifungal screening as follows: Sterile Sabouraud Dextrose Agar was inoculated with spores (10^6 spores/ml) and distributed into Petri plates of 90 mm diameter. The disc size used was sterile 6 mm Whatman No. 40 filter paper. Under aseptic conditions, the discs are placed on the agar plates and then 5 µl from the fennel seeds essential oil are put on the discs. The plates are incubated at 30°C for 2 to 7 days. Antifungal activity is assessed by measuring the diameter of the growth-inhibition zone in millimeters (including disc diameter of 6 mm) for the test organisms comparing to the controls.

Microatmospheric method

The antifungal activity of the seeds' essential oil of fennel against fungi is undertaken using inverted Petri plate described by Singh et al. (2006) with some modification. PDA plates are prepared using 90 mm Petri dishes containing 20 ml of PDA. A 6 mm (diameter) agar disc of each fungus was cut from the periphery of the active growth culture (2 to 7days old) and the mycelial surface is placed upside down on the centre of the dish. The Petri dish was then inverted and a small paper disc (6 mm diameter, Whatman No. 40) was placed inside on the lid of each Petri dish. An aliquot amount 5 µl of essential oil was applied to the paper disc. Incubation of the fungus and test was conducted in a growth chamber at 30°C. Each test was replicated for three times. The antifungal activity was determined after 2 to 14 days incubation by means of the percentage of inhibited redial growth as shown in the following equation:

$$AI_{vap}\ (\%) = [(D_C - D_S) / D_C] \times 100$$

D_S, the diameter of growth zone in the experimental dish (mm); D_C, the diameter of growth zone in the control dish (mm).

Broth dilution method

The minimum fungistatic and fungicide concentration was determined by broth dilution method described by Bajpai et al. (2008) and Bajpai and Kang (2010) from the original method of Murray et al. (1995), with some modification. A 10 µl spore suspension (10^6 spores/ml) of each test fungus was inoculated into the test tubes in PDB (potato dextrose broth) medium with various concentrations (10 000, 5 000, 2 500, 1 250, 625, 312.5, 156.25, 78.125 and 39.06 µg/ml). The mixture, constituting the PDB, spore suspension and essential oil, was homogenized and incubated at 30°C for 2 to 7 days. The control tubes containing PDB medium are inoculated only with spore suspension. The MFCC and MFSC are expressed in µg/ml.

Agar dilution method

Antifungal index (AI_{50}) of fennel seeds' essential oil is determinate using the agar dilution method described by Chang et al. (1999) and Cheng et al. (2006). Briefly, a 6 mm (diameter) agar disc of fungus each removed preceding cultures, are deposited in the center Petri containing 20 ml PDA (potato dextrose agar) with various concentrations of the essential oil of fennel. The testing dishes are incubated at 30°C. When the mycelium of fungi reached the edges of control dishes (without adding essential oils or constituents) for 2 to 14 days, the antifungal indices are calculated. Each test is repeated three times, and the data averaged. The AI_{50} values (the concentration in µg/ml that inhibited 50% of the mycelium of fungi growth) are calculated. The formula of antifungal indices is shown as follows:

$$AI\ (\%) = [(D_C - D_S) / D_C] \times 100$$

D_S, the diameter of growth zone in the experimental dish (mm); D_C, the diameter of growth zone in the control dish (mm).

RESULTS AND DISCUSSION

Essential oil yield

Obtained essential oil has pale yellow color with an aromatic odor. Only small quantities were recovered, **the** yield obtained is close to 0.79 ± 0.02%. This is lower than those quoted by the literature. In general, the essential oil

Table 1. Inhibition zone (mm) of fennel seeds' essential oil against test fungus.

Fungus	Seeds' EO of fennel (5 µl/disk)
Alternaria	20.67 ± 1.527
Aspergillus fumigatus CIP 1082.74	17.33 ± 1.528
Aureobasidium	ND
Fusarium	21.67 ± 2.082
Penicillium	22.67 ± 0.577
Rhizopus	18.33 ± 0.577
Trichophyton rubrum CIP 2043.92	17.33 ± 2.082

ND: not determined.

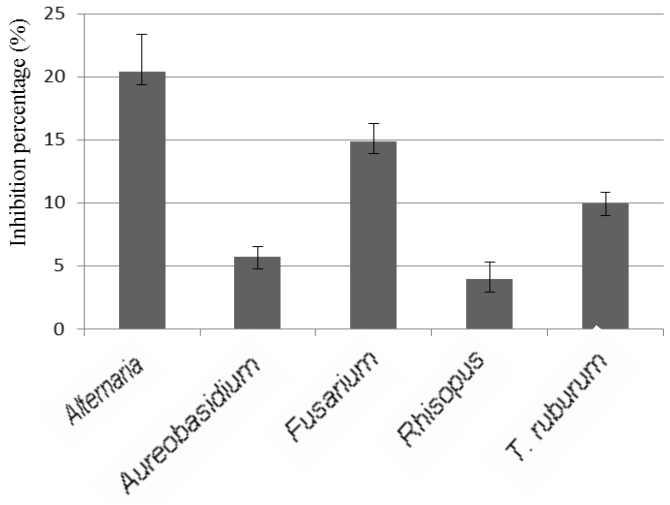

Figure 1. Inhibiting effect of the vapor of fennel seeds' essential oil (5 µl/boite) on mycelia growth.

yield of fennel seeds varies from 2.5 to 6% with an average of 3.5% (Garnéro, 1996).

Sensitivity of fungus strains to the essential oil of fennel seed

The diffusion method enables us to highlight antifungal capacity of seeds' essential oil of fennel against the fungus strains tested. Our results indicate that the essential oil of fennel seed has an inhibiting capacity of the mycelia growth of all the strains tested excluding the *Aureobasidium* spp. strain whose inhibition zone is not given. The inhibition zones are summarized in Table 1. They vary between 17 and 22 mm (including the diameter of disk of 6 mm).

The disk method is limited only to the screening of the antifungal activity because the capacity of the discs and the zones of inhibition are limited. In the present study, our applied method does not enable us to make a comparison between the strains tested which are not representative. Consequently, the calculation of inhibition diameters is not exact; it is the case of *Aureobasidium* spp., where the diameters of inhibition are not calculable. Sharma and Tripathi (2007) have proved that the antifungal activity of essential oils can be evaluated by the dilution method better than by the diffusion method, since in the latter, the size of the zone of inhibition depends on the diffusion of the insoluble compounds in the culture medium. The same results are reported by Hammer et al. (2003). Consequently, the evaluation of the antifungal activity with this method is insufficient; it is thus necessary to use other techniques such as the incorporation of essential oil directly in the culture medium. In the method of dilution, the only disadvantage is that it requires a big quantity of EO.

Sensitivity of fungi strains to the volatile compounds of essential oil of fennel seeds

The antifungal activity of the volatiles compounds of essential oil of fennel seeds is determined by the reversed plate method. The vapor of essential oil of fennel seeds has showed an inhibiting activity which is not negligible with an amount of 5 µl. All the fungi strains are inhibited by the vapor of the essential oil of fennel seed with 5 µl/plate but with a different rate of inhibition according to the strain. *Rhizopus* and *Aureobasidium* are the strains most sensitive to the essential oil of fennel seed with inhibition rates of 18.33 ± 0.577 and 17.33 ± 1.528%, respectively (Figure 1).

In spite of the existence of many reports on the antifungal activity of essential oils by direct contact method, we have noted that information on the antifungal activity of the volatile phase of essential oil is rare. The volatile compounds of essential oil of fennel seeds are endowed with antifungal activity. In the present study, the vapor of essential oil of fennel seeds has showed an inhibiting activity which is not negligible with an amount of 5 µl/plate.

Certain researchers have found that the essential oil of fennel seeds is effective by the method of reversed plate than by the direct contact method. They posited that the lipophilic nature of essential oils make them more

Table 2. Minimum fongistatic concentrations (MFSC) and Minimal fungicidal concentrations (MFCC) of fennel seeds' essential oil.

Fongus strain	MFSC (µg.ml^{-1})	MFCC (µg.ml^{-1})	MFCC/ MFSC
Alternaria	625	1250	2
Aspergillus fumigatus CIP 1082.74	1250	1250	1
Aureobasidium	625	1250	2
Fusarium	1250	1250	1
Penicillium	1250	1250	1
Rhizopus	1250	1250	1
Trichophyton rubrum CIP 2043.92	1250	1250	1

Table 3. Recapitulation of the antifungal indices (AI_{50}) of fennel seeds' essential oil.

Fungus strain	AI_{50} (µg.ml^{-1})
Alternaria	26.22 ± 0.693
Aspergillus fumigatus CIP 1082.74	ND
Aureobasidium	29.17 ± 2.021
Fusarium	1767 ± 39.589
Penicillium	ND
Rhizopus	304.84 ± 13.012
Trichophyton rubrum CIP 2043.92	170.16 ± 4.458

ND: non given.

absorbable by the fungi mycelium than by the hydrophilic nature (Soylu et al., 2005). The same method is used by Singh et al. (2006); they found that the essential oil of fennel seeds has an inhibition rate of 100% against *Aspergillus niger* and *Aspergillus flavus* with an amount of 6 µl/disk, it proved strongly effective even with 4 µl for *A. niger*. While being based on the results of the microaromatogrammes, the antifungal activity of essential oil of fennel seeds could be due to the combinative effect of the vapor of essential oil and the direct contact.

Determination of the MFSC and MFCC

These concentrations are measured with an aim of defining the borders of the sensory acceptability and the antifungal effectiveness of essential oils (Tiwari et al., 2009). The values of MFSC and MFCC of seeds essential oil extracted from fennel are presented in Table 2.

Aligiannis et al. (2001) proposed a classification of the natural extracts, based on the results of the values of MFSC, as follows: strong activity: CMFS is lower or equal to 500 µg/ml; moderate activity: MFSC between 600 and 1500 µg/ml and weak activity: MFSC higher than 1600 µg/ml. While basing on this classification, the essential oil of fennel seeds seems to have a moderate activity.

Recently, several researchers have reported that the oxygenated terpenoides and their derivatives are the principal components of essential oils; these compounds extremely have an inhibiting potential on pathogenic microbial strains (Hossain et al., 2008). Purified compounds derived from the essential oil of fennel, like the anethol, are already studied to test their antimicrobial activities (De et al., 2002).

Determination of the antifungal indices (AI_{50})

To obtain concentrations more precise than the MFSC and MFCC, another parameter is given: the AI_{50}, which is the concentration which inhibits 50% the mycelia growth. All the concentrations of the essential oil of fennel seeds applied prevented, partially or completely, the growth of the fungi strains tested. The AI_{50} gives graphically and recapitulates in Table 3.

According to the results obtained, it is clear that the strains tested do not have the same AI_{50}, the strains belonging to the *Alternaria* and *Aureobasidium* kinds is most sensitive. The seeds' essential oil of fennel inhibits the total growth (100%) of *Aureobasidium* kinds with 2000 µg/ml and 4000 µg/ml the *Alternaria* stains. On the other hand the strains belonging to the *Fusarium* and *Rhizopus* kinds are the most resistant strains. According to Wiley (2005), the color characteristic of much of the fongus is due to the pigmentation of conidiums. Consequently, the change of the color of *A. fumigatus*, *T. rubrum*, *Alternaria* and *Aureobasidium* could be due to the effect of the essential oil of fennel seeds on conidiums.

The variation of the values of the AI_{50} is due probably to the nature of the wall of the fungi strains which is composed of a complex network of proteins and polycarbohydrates, and which varies in composition according to the fungi species. The disturbance of this matrix can have like consequence a defective wall, which becomes sensitive to lyses osmotic and sensitive with the antifungal agents (Yen and Chang, 2008).

The mechanism of action of essential oils against fungi strains, until now, is not completely understood, but some authors gave several assumptions according to their observations. The majority of the studies on the

mechanism of action of essential oils are accentuated on their effects on the cellular membranes. In fact, the active compounds attack the wall and the cellular membrane, affecting of this fact, the permeability and the release of the intracellular components, while also interfering with the function of the membrane (Chalchat et al*.,* 1997; Bang et al., 2000; Carmo et al., 2008).

According to the observations of Soylu et al. (2005), the essential oil of *F. vulgare* Mill. involved deteriorations of the morphology of the hypha; they also observed large blisters inside the cellular wall. In most of the cases, the mycelium cells do not have any more or exhausted cytoplasm of the organelles. Similar observations with other essential oils have been reported by Fiori et al. (2000). Moreover, the antifungal activity of essential oils could also be related to the interference of the components of essential oil in enzymatic reactions of synthesis of the cellular wall, which affects the fungi growth (Chalchat et al., 1997). These suggestions have already been reported by Bang et al. (2000). They have studied the inhibition of the enzymes synthesizing the wall of the fungi cells by examining the inhibiting effects of essential oil on β-(1,3)-glucan and chitin synthases. Chalchat et al*.* (1997) announced that essential oils damage a series of enzymatic systems of the fungi, thus affecting the synthesis and the energy production structural components.

The toxicity of solvent can be criticized because the solvent should not prevent the biological process. Attention should also be given to the possible interactions between the dissolved solvent and bodies, while the solvent reacts with some compounds to produce complexes or to cause the decomposition, dehydration, or the isomerization of these compounds (Mohammedi, 2006).

Conclusion

Our results indicate that the essential oil of fennel seeds has an interesting antifungal activity. According to our results, we can propose the essential oil of fennel seeds like an alternative to the synthetic antifungal agents for food industries. Consequently, it would be also interesting to test the essential oil of fennel seed in food to develop new antifungal agents to prevent or delay the deterioration of the foodstuffs.

The use of essential oils as antifungal agents shows two main features: on the one hand, they are natural, therefore, are surer for the consumers, on the other hand, they present several action modes of antifungal what would prevent the adaptability of the microorganisms. With the rise of this study, it would be interesting to undertake a thorough study on essential oils of fennel seeds in order to insulate, purify and identify the active ingredients (having an antifungal activity) of this plant. Other tests on antifungal ability in food are necessary in order to develop these natural products for food industries. We think that it is necessary to exploit the great diversity of the aromatic plants in particular for their secondary metabolites (essential oils, polyphenolic compounds) and to subject them to true scientific investigations, in particular in food industries.

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REFERENCES

Aligiannis N, Kalpotzakis E, Mitaku S, Chinou IB (2001). Composition and antimicrobial activity of the essential oils of two *Origanum* species, J. Agric. Food Chem., (40): 4168-4170.

Bajpai VK, Shukla S, Kang SC (2008). Chemical composition and antifungal activity of essential oil and various extract of *Silene armeria* L, Bioresou. Technol., (99): 8903–8908.

Bajpai VK, Kang SC (2010). Antifungal activity of leaf essential Oil and extracts of Metasequoia glyptostroboides Miki ex Hu, J. Am. Oil. Chem. Soc., (87): 327–336.

Bang KH, Lee DW, Park HM, Rhee YH (2000). Inhibition of fungal cell wall synthesizing enzymes by trans-cinnamaldehyde, Biosci. Biotechnol. Biochem*.,* (64): 1061-1063.

Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods: a review, Int. J. Food Microbiol., (94): 223-253.

Carmo ES, Lima EDO, De Souza EL (2008). The potential of *Origanum vulgare* l. (Lamiaceae) essential oil in inhibiting the growth of some food-related *Aspergillus* species, Brazilian J. Microbiol., (39): 362-367.

Chahardehi AM, Ibrahim D, Sulaiman SF (2010). Antioxidant, Antimicrobial Activity and Toxicity Test of Pileamicrophylla, Int. J. Microbiol., ID 826830, p.6.

Chalchat JC, Garry PR, Menut C, Lamaty G, Malhuret R, Chopineau J (1997). Correlation between chemical composition and antimicrobial activity. VI. Activity of some African Essential Oils, J. Essential oil Res., (9): 67-75.

Chang ST, Wang SY, Wu CL, Su YC, Kuo YH (1999). Antifungal compounds in the ethyl acetate soluble fraction of the extractives of Taiwania (*Taiwania cryptomerioides* Hayata) heartwood, Holzforschung, (53): 487-490.

Cheng SS, Liu JY, Hsui YR, Chang ST (2006). Chemical polymorphism and antifungal activity of essential oils from leaves of different provenances of indigenous cinnamon (*Cinnamomum osmophloeum*), Bioresour. Technol., (97): 306-312.

Clevenger JF (1928). Apparatus for the determination of volatile oil, J. Am. Pharm. Assoc., (17): 345-349.

De M, De AK, Sen P, Banerjee AB (2002). Antimicrobial properties of star anise (*Illicium verum Hook f*). Phytother. Res., (16): 94–95.

Dung NT, Kim JM, Kang SC (2008). Chemical composition, antimicrobial and antioxidant activities of the essential oil and the ethanol extract of Cleistocalyx operculatus (Roxb.) Merr and Perry buds, Food Chem. Toxicol*.,* (46): 3632–3639.

Fiori ACG, Schwan-Estrada KRF, Stangarlin JR, Vida JB,. Scapim CA, Cruz MES, Pascholati SF (2000). Antifungal activity of leaf extracts and essential oils of some medicinal plants against *Didymellla bryoniae*, J. Phytopathol., (148): 483-487.

Gachkar L, Yadegari D, Rezaei MB, Taghizadeh M, Astaneh SA, Rasooli I (2006). Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils, Food Chem., (102): 898-904.

Garnéro J (1996). Huiles essentielles. Techniques de l'Ingénieur, traité Constantes physico-chimiques ; K 345-1, p.39.
Hammer KA, Carson CF Riley TV (2003). Antifungal activity of the components of Melaleuca alternifolia (tea tree) oil, J. Appl. Microbiol., (95) : 853–860.
Hossain MA, Ismail Z, Rahman A, Kang SC (2008). Chemical composition and anti-fungal properties of the essential oils and crude extracts of *Orthosiphon stamineus* Benth, Indust. Crops Prod., (27): 328–334.
Mohammedi Z (2006). Etude du pouvoir antimicrobien et antioxydant des huiles essentielles et des flavonoïdes de quelques plantes de la région de Tlemcen. Thèse magistère, Université Abou Bakr Belkaïd Tlemcen, p.155.
Moll M (1998). Additifs alimentaires et auxiliaires technologique. Ed. DUNOD. Paris,pp. 89-99.
Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolke RH (1995). Manual of clinical microbiology. ASM, Ishington Vol (6). ASM, Washington.
Nakahara K, Alzoreky NS, Yoshihashi T, Nguyen HTT, Trakoontivakorn G (2003). Chemical Composition and Antifungal Activity of Essential Oil from *Cymbopogon nardus* (Citronella Grass). JARQ, 37(4): 249-252.
Ownagh A, Hasani A, Mardani K, Ebrahimzadeh S (2010). Antifungal Effects of Thyme, Agastache and Satureja Essential Oils on *Aspergillus fumigatus, Aspergillus flavus* and *Fusarium solani,* Veter. Res. Forum, (2): 99-105.
Özcan MM, Chalchat JC (2004). Aroma profile of *Thymus vulgaris L*. growing wild in Turkey, Bulg. J. Plant Physiol., 30(3-4): 68-73.
Özcan MM, Chalchat JC (2006). Effect of collection time on chemical composition of the essential oil of *Foeniculum vulgare* Subsp. *piperitum* growing wild in Turkey. Eur. Food Res. Technol., (2006) 224: 279–281.
Rashid A, Qureshi MZ, Raza SA, William J, Arshad M (2010). Quantitative determination of antioxidant potential of *Artemisia persica*. Analele UniversităŃii din Bucureşti – Chimie (serie nouă), 19(1): 23-30.
Rasooli I, Fakoor MH, Yadegarinia D, Gachkar L, Allameh A, Rezaei MB (2008). Antimycotoxigenic characteristics of *Rosmarinus officinalis* and *Trachyspermum copticum* L. essential oils, Food Chem., pp.135-140.
Rozier J, Carlier V, Bolnot F (1986). Bases microbiologiques de l'hygiène des aliments. Éd. SAPALC. Paris,pp. 130-143.
Sharma N, Tripath A (2007). Effects of *Citrus* (L.) Osbeck epicarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem. Microbiol. Res., 163(3): 337-344.
Singh G, Maurya S, De Lampasona MP, Catalan C (2006). Chemical constituents, antifungal and antioxidative potential of *Foeniculum vulgare* volatile oil and its acetone extract, Food Control, (17): 745-752.
Soylu EM, Yigitba H, Tok FM, Soylu S, Kurt S, Baysal Ö, Kaya AD (2005). Chemical composition and antifungal activity of the essential oil of *Artemisia annua* L. against foliar and soil-borne fungal pathogens, J. Plant Dis. Protect., 112 (3), 229–239.
Tiwari BK, Valdramidis VP, O'Donnell CP, Muthukumarappan K, Bourke P, Cullen PJ (2009). Application of Natural Antimicrobials for Food Preservation, J. Agric. Food Chem., (57): 5987–6000.
Wang HF, Yih KH, Huang KF (2010). Comparative Study of the Antioxidant Activity of Forty-five Commonly Used Essential Oils and their Potential Active Components. J. Food Drug Anal., 18 (1): 24-33.
Wiley J (2005). Essential microbiology. Southern Gate, Chichester, West Sussex PO19 8SQ, England, p. 481.
Yen TB, Chang ST (2008). Synergistic effects of cinnamaldehyde in combination with eugenol against wood decay fungi, Bioresouc. Technol., (99): 232-236.