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## Using of Thymol for Export Preservative Fruits from Spoilage to the using from Consumer

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**Abstract:** Isolates of fungi were gathered from imported and damaged fruits of grapes, as well as orange and grapefruits respectively which were purchased from local markets. Then extracted thymol complex from thyme plant, then tested its frustration effect in growth some isolated fungi such as *Fusarium graminearum* which caused grape damage, and *Penicillium nalgiovense* which caused orange and grapefruit damage. Then explain that concentration of the extracted thymol from thyme plant (0.5 mg/ml) in the growth culture is killing for *F. graminearum* fungi, and (1.5mg/ml) concentration from growth culture is killing for *P. nalgiovense* fungi, normally the thymol complex become mutation and poison in the high concentration, and According to my previous study about the mutation effect for the thymol in fungal conidia *Aspergillus amstelodami* that the concentrations (25, 30 mg/ml) shows a mutation effect, after high repetition higher than the concentrations (25, 30 µg/ml) shows a mutation effect, after high repetition higher than the spontaneous mutation for null manipulated (negative control), while the low concentrations (10, 15, 20 µg/ml) not has any mutation effects.



For more safety we advise that use it to saving the fruits and crops which peeling its external capsule such as citric to avoid damage them during shipping, storage and exportation.

Key words: *Fusarium graminearum*, *Penicillium nalgiovense*, thyme, thymol, preservative fruit and crops.

## استخدام مركب الثايمول Thymol لحفظ الفواكه المصدرة من التلف حين استخدامها من قبل المستهلك

د. مها أكرم الرجوب

قسم علوم الحياة / كلية العلوم / جامعة الموصل

### الملخص:

تم جمع عزلات فطرية من ثمار العنب والبرتقال والسندي المستوردة والتالفة من الأسواق المحلية في مدينة الموصل (العراق)، وتم استخلاص مركب الثايمول من نبات الزعتر ومن ثم اختبار تأثيره الشببي في نمو بعض الفطريات المعزولة والتي تسببت في تلف تلك الثمار ومن هذه الفطريات *Fusarium graminearum* المسبب لتلف العنب والفطر *Penicillium nalgiovense* المسبب لتلف البرتقال والسندي، وتبين أن تركيز الثايمول المستخلص من نبات الزعتر بمقدار 0.5 ملغم / مل من وسط النمو هو تركيز قاتل للفطر *F. graminearum* وإن التركيز 1.5 ملغم / مل من وسط النمو هو تركيز قاتل للفطر *P. nalgiovense* المعزول من البرتقال والكريغوت (السندي). وبما أن مركب الثايمول يصبح مطفراً وساماً في التراكيز العالية وحسب دراستي المشتركة السابقة حول التأثير التطفيري لمركب الثايمول في كونيديات الفطر *Aspergillus amstelodami* أظهرت التراكيز 25 و 30 مايكروغرام / مل تأثير تطفيري وبتكرار عالي أعلى من تكرار الطفرات التلقائية لمعاملة الصفر (السيطرة السالبة) بينما التراكيز الواطئة منه 10، 15 و 20 مايكروغرام / مل لا تظهر تأثيراً تطفيرياً تجاه كونيديات الفطر فلأمان ينصح باستخدام مركب الثايمول بتراكيز واطئة لحفظ المحاصيل والفواكه التي يزال غلافها الخارجي كالحمضيات لمنع تلفها أثناء الشحن والخزن والتصدير.



الكلمات المفتاحية : نبات الزعتر . الشامبول . *Penicillium* . *Fusarium* *graminearum* . *nalgiovense* . مادة حافظة . الفواكه والمحاصيل .

## Introduction

Some fruits effected with spoilage early or when they stay for a long time before using. The delay in crops marketing because of these crops are surplus in those areas, so exported to other areas nearby or far from its areas, accelerate of spoilage them that they affected with fungi, bacteria, or insect disease and another injures which caused the early spoilage for exporting fruits, which caused huge wastages. Current fungi in the fruits belong for two genus *Fusarium* and *Penicillium* like *Penicillium italicum wehner*, *P. digitatum cass*, and *Phytophthora citrophthora* (Al- Shukri, 1994). So many researchers try to use preservative matters for keep fruits faraway from spoilage for bigger period, so we try to extract (thymol) from thyme plant *Thymus spp* it is wild plant spread in our countries(Iraq) in a large form. As well as, we see that study of some fungi which caused the crops spoilage especially the fruits which are peeling like the exporting citrus and kill them by using the thymol complex which is regard a Phenol complex which has high inhibition effect against the fungi ,the essential oils of thyme (*Thymus vulgaris* L.) inhibitory effect as highest antifungal activity aganus *Aspergillus niger* (200 ppm) and they noticed from GC and GC-MS thymol concentrate 30.91% from the thyme oil (Noshirvani and Fasihi,2018), Where I was studied previously in doctorate dissertation (Al- Rejaboo, 2004) effect of thymol complex which extracted from thyme plant towards some fungi, where the extract frustrated 100% growth of the fungi *Aspergillus fumigatus* and *A. niger* on (1.5 mg/ml) concentration from the culture (two fungi isolated from chronic suppurative otitis media), the extract frustrated 100% growth of



*Trichophyton mentagrophytes* which isolated from skin scraping on (0.5 mg/ml) concentration from culture, the extract frustrated 100% growth of isolated *A. flavus* from corn seeds on (1 mg/ml) concentration from culture, but the extract frustrated 100% growth of isolated *A. niger* fungi from rice plant on (1 mg/ml) concentration.

So, we can see that thymol complex has a high frustration effect towards many fungi, therefore we see study its frustration effect towards the fungi caused fruits damage such as orange, grapefruit, and grape. According to my previous study about the mutation effect for the thymol in fungal conidia *Aspergillus amstelodami* that the concentrations (25, 30 µg/ml) shows a mutation effect, after high repetition higher than the spontaneous mutation for null manipulated (negative control), while the low concentrations (10, 15, 20 µg/ml) not has any mutation effects. the high concentrations of this matter (higher than 25µg/ml) regard as a mutation matter (Al- Rawi & Al- Rejaboo, 2010) so must use it as a preservative matter in low concentrations as well as avoid preserved the grains and food in this matter.

## Materials and methods:

### 1- Isolate the fungi :

Isolated randomly some of the pollution and growing fungi on the imported crops which caused its spoilage from these crops orange, grapefruit and grape, where took a sample from these growths in aseptic conditions and inoculated in Petry dish consists of (PDA) (Potato Dextrose Agar) (Pitt and Hocking, 1985), the dishes incubating at  $2\pm 28^{\circ}\text{C}$  for seven days, with continuing the results observe during ocubation period. The growing fungi isolates preserve in slant mediums till the diagnosis ( Koneman.et al,1979;



Mengistu and Sinclair, 1979, Leslie et al, 1990, Kwon- chung and Bennett,1992 and deHoog & Guarro,1995).

## **2- Diagnosis of molds:**

The growing fungi isolates ocubated on the PDA medium (PDA for seven days, and studied the general characterizes for every isolates from some sides like shape, color, and texture of the mediatised, and shape, color, and dimenesios of the conides, as well as used planting on glass slide technique, in addition to taking a sample from every mediatised and inoculated by using technique of the medium inoculation on the three basic medium for diagnosis as follows:

- 1- Czapek Yeast Extract Agar (CYA).
- 2- Malt Extract Agar (MEA).
- 3- 25% Glycerol Nitrate Agar (G25N).

These medium ocubated at 5, 25, 37 C° for seven days (Pitt and Hocking, 1997).

## **3- Extracting of the Thymol from thyme plant:**

Obtaind of the alcoholic extract from leaves and flower blossoms powder for dry thyme plant by using Soxhelet and use Mythanol (MeOH) as a solvent. then filtering the MeOH extract as a normal extracting for avoid the sediments during stages of the extract, and then the output dry by vertigo evaporator and melt in 100 ml from HCL acid in concentration of M 0.02, the grays separated with Chlorophyll rests by Petroleum ether in suitable size by using Separatory Funnal, this step repeated many times, the water layer took and pulled from it the phynol complexes by adding 50 ml from



Diethyl ether (DEE), the layer of (DEE) which enrich with the phynols and organic acids separated and gathering in the glass jug (Sousek et al, 1999). then submitted to hydrolysis operation in the basic media (10% NaOH) under the ebullition and direct increasing for on hour or more according to the sample size, then leave for 24 hours for completing the reaction, and after that equivalence with lighting HCL by observe the variation in the sunflower leave color, and the extracted the phynol and organic complexes by DEE and by using the separating funnel, after that the DEE dry by adding a suitable quantity from non water CaCl<sub>2</sub> and melding good in the separating funnel, so we have two layers, took the DEE layer and measured its concentration by UV Scanning Spectrophotometer, and know its absorbing and Landa max (nm) and compare it with its absorbing and (nm) for trading thymol regard it as Standard (Al- Rejaboo, 2004) according to the following equation:

$$C_n = \frac{A_n C_1}{A_1}$$

Where:

C<sub>1</sub> the knowing matter concentration

A<sub>1</sub> knowing matter absorbing

C<sub>n</sub> ignoring matter concentration

A<sub>n</sub> ignoring matter absorbing

Measured IR for thymol extract.



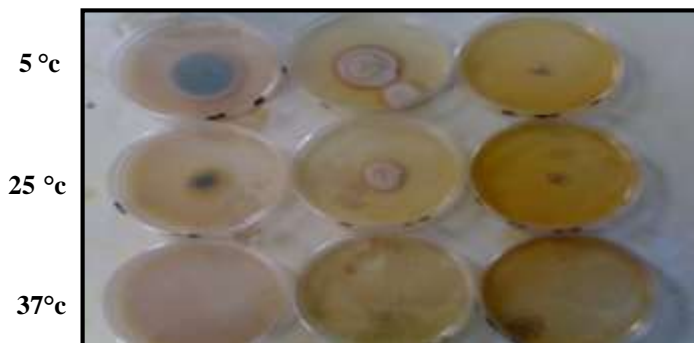
#### 4- Test of Thymol extract effect on the fungi:

Isolated Fungi medium took in age of seven days which incubated at 25 °C under aseptic conditions, take disks in 5mm diameter from the external fungal colony, then every disk put on medium of PDA adding to it thymol extract in concentrations (0, 0.1, 0.5, 1, 1.5, 2, 2.5) mg/ml from the plantation medium where as followed the law  $N_1V_1 = N_2V_2$  for medium those concentrations depending to the standard solution for thymol in concentration 10 mg/ml 1k DDE (Diethyl ether) which aseptic by pasteurization. Used three repeated for every concentration and incubated at 25°C for seven days, the results were recorded.

#### Results and Discussion:

The growing fungi were isolated on some of the spoilage imported fruits which took randomly from the local markets includes orange, grapefruit, and grape, where isolate some fungi from them which belong the genus: *Fusarium* and *Penicillium*, and then diagnosis their species depending on the differential mediums MEA, G25N2 and CYA at 5 °C, 25 °C, 37 °C, for seven days, then note the cultural characteristics and microscopic characterizes for the fungi mediated like shape, color, texture and size, shape of spores depending on the classification key (Pitt & Hocking, 1997).

showing that the fungi is *F. graminearum* which isolated from the grape., and the fungi *P. nalgiovense* which isolate from orange and grapefruit as showing in the picture (1), also, the diagnosis of these two fungi depended on glass slide technique.







### Picture (1)

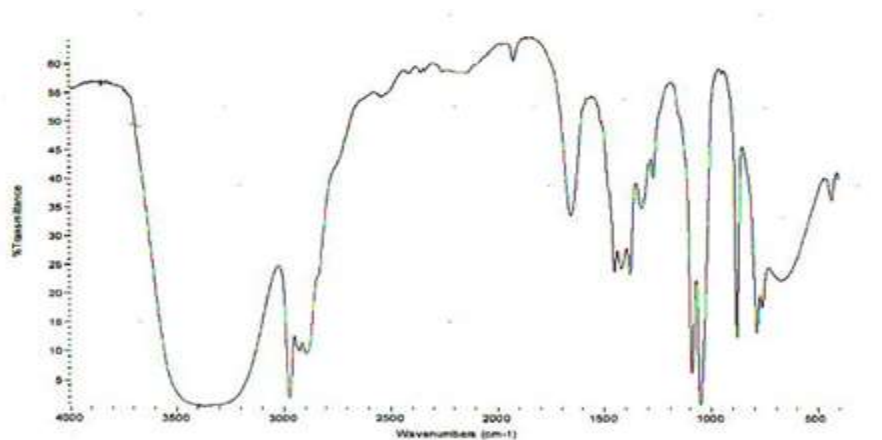
Diagnosis of fungi *Pencillium nalgiovenese* by differential media(CYA,G25N,MEA) at 5, 25,37 C° for seven days.

Thymol complex extracted from thyme plant and discovering by using spectrums IR figure (B-1) see that thymol which existence in the thyme is a mixture from raw thymol with its ester derivations (ester groups show absorbing in (1730cm<sup>-1</sup> – 1745 cm<sup>-1</sup>) table (1), so, when study its frustration effect towards fungi subject of the study, didn't show any action towards it, after carrying out the hydrolyses in the basis medial of the extract, the absorbing which belong to the ester carbonelic group was disappear (figure c-1), it is same to the commercial thymol spectrum (A-1), we can see values absorbing of IR spectrum for each of commercial thymol and extract from the thyme plant before operation of the hydrolysis purifying and after it (table 1), after this using absorbing spectrums of U.V. as shown in figure (2)

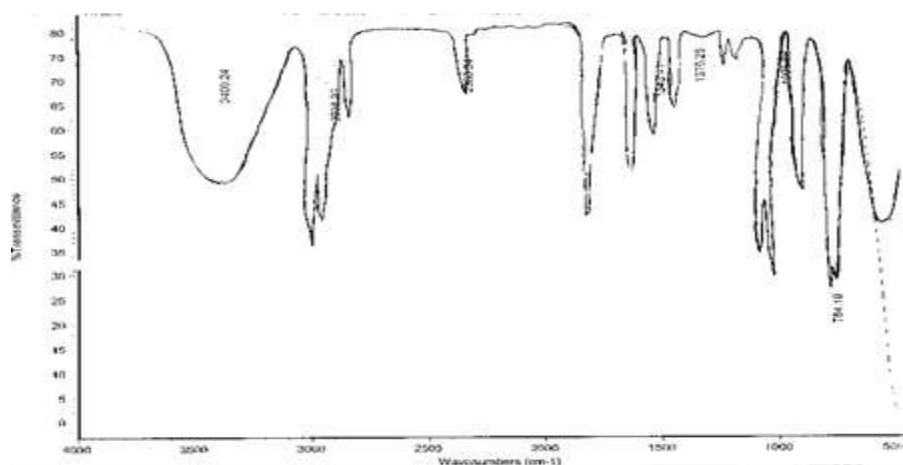




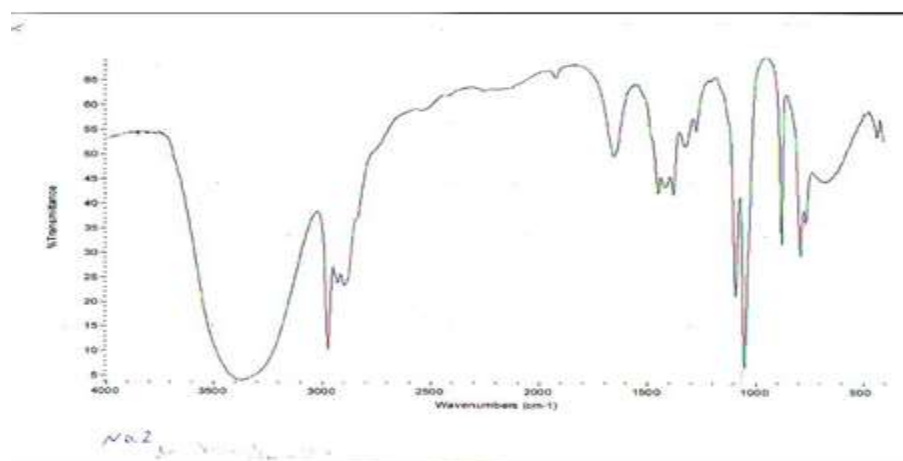
(A)  
absorption



(B)  
absorption



(C)  
absorption





Figuer(1) :IR absorbing:

(A) commercial thymol.

(B) extracted thymol from thyme before hydrolysis and purifying.

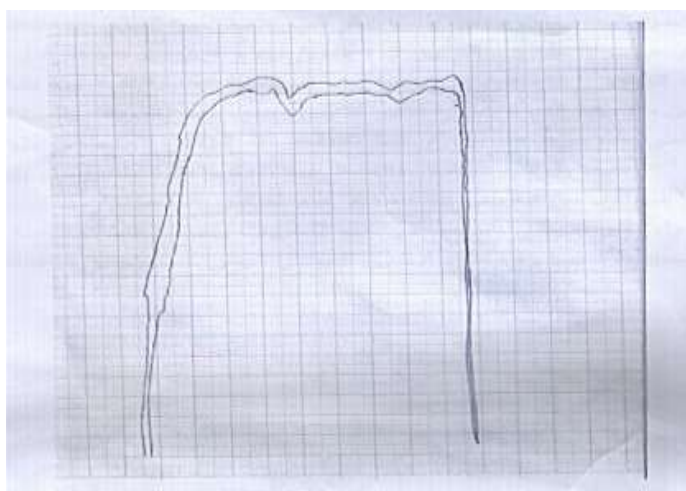
(C) extracted thymol from thyme after hydrolysis and purifying.

Table (1): Values of absorbing spectrum IR for each of: commercial thymol (standard), extracted thymol from thyme before and after hydrolysis and purifying.

values of absorbing (cm <sup>-1</sup> )							Thymol source
Absorb ing CO-	Absorb ing CO <sub>2</sub> -	Aromatic epithit	Curving C-O	Typical absorbin g for Benzene ring	v CH-	v O-H	
-	-	790-880	1050-1100 Split absorbing indicative on ether group C – O /	1676-1480-1400	2990-2850 Strong band	3450-3500 Wide band	Thymol Commercial (standard)
1745 Carbon yl group CO-	2400 Middle severity band for CO <sub>2</sub> -	750-850	1050-1100 Split absorbing indicative on ether group C – O /	1580-1490-1430	2992-2860 Strong band	3450-3500 Wide band	Thymol Extracted from thyme plant after purifying operation



-	-	790-890	1050-1100 Split absorbing indicative on ether group C – O /	1660-1580-1480-1393	3000-2900	3400-3500 Wide band	Thymol Extracted from thyme plant before purifying operation 1
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Figure(2)  
U.V. Scanning spectrophotometer by using DEE solvent

Up curve is the extract thymol

Down curve is the commercial thymol

To protect the crops from spoilage during the transportation and marketing till reach to the consumers, then test effect of the thymol extract on the two fungi for possibility of using as a preservative matter, the table



(2) showing that thymol extract concentration in 0.5 mg/ml from media didn't show fungi grow comparing with diameter of the standard colony 8,4 cm, while the diameter of the fungi colony 5,8 cm at concentration 0.1 mg/ml for the fungi *F. graminearum*, while the fungi *P. nalgiovense* which isolated from orange the diameter of the colony was 0 cm at the 1.5 mg/ml and concentration 2.6 cm at concentration of 1 mg/ml, 5.3 cm at the concentration of 0.1 mg/ml while diameter of the comparing colony is 5.6 cm, while diameter of the fungi colony *P. nalgiovense* which isolated from grapefruit 0 cm at concentration of 1.5mg/ml and 2.4 cm at concentration of 1mg/ml and 3.6 cm at the concentration of 0.1 mg/ml, while diameter of the comparison colony (standard colony) is 5,8 cm as showing in the picture (2). From observe the percentage of effect thymol on these fungi which showing in the table (3) we see that the fungi *F. graminearum* inhibited 100% at 0.5mg/ml concentration, the fungi *P. nalgiovense* which isolated from orange inhibited 100% at 1.5mg/ml concentration, and *P. nalgiovense* which isolated from grapefruit inhibited 100% at 1.5mg/ml concentration. From here we can see that the thymol extract has clear inhibitory effect on these fungi at little concentrations especially towards the fungi *Fusarium*, and saw that there no big difference in the reacting of two fungi isolates *P. nalgiovense* toward the thymol extract.



Table (2)  
Effect of the thymol extracting on average of isolated fungi mediatises diameters

2.5	2.0	1.5	1.0	0.5	0.1	comparison +DEE	comparison	Thymol con. (mg/ ml)  Fungi Name
0	0	0	0	0	5.8	8.4	8.4	<i>Fusarium graminearum</i> Isolated from Grape
0	0	0	2.6	4.3	5.3	5.6	5.6	<i>Penicillium nalgioense</i> Isolated from Orange
0	0	0	2.4	2.8	3.6	5.8	5.8	<i>Penicillium nalgioense</i> Isolated from Grapefruit

Table (3)  
Percentage of thymol effect on the isolated fungi from fruits

2.5	2.0	1.5	1.0	0.5	0.1	comparison n + DEE	comparison n	Thymol Con. (mg/ ml)  Fungi Name
% 100	% 100	% 100	% 100	% 100	% 31	% 0	% 0	<i>Fusarium graminearum</i> Isolated from Grape



% 100	% 100	% 100	% 53.6	% 23.3	% 5.35	% 0	% 0	<i>Penicillium nalgiovense</i> Isolated from Orange
% 100	% 100	% 100	% 58.7	% 51.8	% 38	% 0	% 0	<i>Penicillium nalgiovense</i> Isolated from Grapefruit

Picture (2)

Inhibitory effect of thymol extract on fungi *Pencillium nalgiovenese* which isolated from orange





Bennis and others (2004) found that mechanism of thymol inhibitory for fungi were in causing damages in the external shape for fungi cells where caused in cracks the cellular walls for the fungi *Saccharomyces cerevisiae* through observe clear cracks on the cellular walls for the fungi by electronic microscope, as well as, (Al-Rejaboo, 2004) found that the thymol extract from thyme has high inhibitory effect towards many fungi such as: *Aspergillus flavus*, *A. fumigates*, *A. niger*, *Candida albicans*, and *Trichophyton mentagrophytes*, , and point out that the effect was in pressing composing some proteins of the cellular wall, some researchers assuring that the thymol has high inhibitory effect towards some fungi (Pier et al, 2008), the researcher Sokovic and others (2009) mentioned that thymol has high inhibitory effect towards fungi *Fusarium tricinctum*, and Barrera and others (2009) noticed that thyme oil and thymol extract which extracted from it regard as a good fungi anti- biotic for fungi *F. oxysporum*, Dambolena and others (2008) found that thymol has a strong inhibitory effect towards *F. verticillioides*, Korukluoglu and others (2009) that basil oil consist of mainly from thymol and it was strong in frustration towards fungi *F. semitectum*, Vazquez and others (2001) noticed that thymol frustrated growth of fungi *Penicillium citrinum* in the culture and in the cheese products, Klaric and others (2007) pointed out that thymol and its oil frustrated growth of *Penicillium* in concentration lower than 20 microgram/ ml from the culture, observed that thymol which extracted from thyme oil or basil oil which rich in thymol have strong inhibitory effect towards species of *Penicillium* especially those which caused citrus spoilage, then found that strongest oil anti fungi especially *Penicillium* is thyme oil (Arras and Usai, 2001, Ameziane et al, 2007, Svirev et al, 2007, Koruklouglu, et al, 2009 and Sokovic et al, 2009;).





And then found that thymol has inhibitory effect for some isolated fungi from the crops and food products as well as frustrated its vitality acting and its product for fungi toxin especially *Aspergillus flavus* A. *parasiticus* this is useful in decreasing production of Aflatoxin poisons by the fungi which polluted crops and ensuring its safety during transportation and storage till reaching to the consumers.( Buchanan and Shepherd, 1981, Mahmoud, 1994, Couladis.et al,2004 and Razzaghi. et al, 2008).

Braga and others (2007) and Liolios and others (2009) pointed out that chance of using thymol as a preservative matter for food products and crops, where noticed that high frustration acting towards fungi of *Candida* and *Listeria monocytogenes* which isolated from food and crops, but observed that thymol has side damages when increase its concentration, where become as a mutation matter by its effect on repeated and duplicate DNA (Mandel, et al, 1985, Gringauz, 1987,), and caused some chromosomes deformations in rat bone marrow when use it in certain concentrations and for long periods (Sebile, 2008), and its using in huge quantities for a long periods caused the liver decay, where effect negatively on the animal cells metabolism (Baudoux, 2000, Al- Nouamy, 2004 and Al-Rawi and Al-Rejaboo, 2010) found that using thymol complex in concentrations of 25 microgram/ml and up may be mutate and poisonous, must care in using this matter as a preservative matter from the concentration and kind of the food product, fruits which peeling can use thymol in lower concentrations for preserving from spoilage and avoid use it for preserve the fruits which have thin teguments and grains which girding directly like wheat, barley and rice, and must take care that thymol complex is a basic component for some evaporating oils which product by the aromatic plants include thyme where its percentage in the oil from 36% to 55% (Manou et all, 1998 and Bruneton,



1999), the essential oils of thyme (*Thymus vulgaris* L.) inhibitory effect as highest antifungal activity against *Aspergillus niger* (200 ppm) and they noticed from GC and GC-MS thymol concentrate 30.91% from the thyme oil (Noshirvani and Fasihi, 2018), as well as the basil and many medical, pharmaceutical and food grasses, therefore must study safety of these grasses and take care in using thymol complex as a preservative matter.

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