# Effect of Essential Oils Extracted from the Peels of Two Species of *Citrus* on Some Fungi

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## Abstract

This study investigated the effect of essential oils extracted from peel of *Citrus limon* and *Citrus reticulata* on two species of fungui: *Penicillium expansum* and *Fusarium proliferatum* and also effect of two fungicides: Hymexazol and Benomyl against this fungi. Results showed that the essential oils of *C. limon* inhibited the radial growth of *P. expansum* and *F. proliferatum* at concentration 4.5 and 5%, respectively. However, the essential oil of *C. reticulate* inhibited this growth at concentration 5.5 and 6%, respectively. Moreover, the two fungicides inhibited radial growth of this fungi. In conclusion, there is a positive relationship between the increasing of concentration and the percentage of inhibiting of radial growth of fungi.

**Keywords:** Essential oils, citrus peels, fungi.

## تأثير الزيوت الطيارة المستخلصة من قشور نوعين من الحمضيات في بعض انواع الفطريات

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**خلاصة**

هدفت الدراسة الحالية فحص تأثير الزيوت الطيارة المستخلصة من قشور نبات الليمون *Citrus limon* واللالنكي *Citrus reticulata* في نوعين من الفطريات (*Penicillium expansum* و*Fusarium proliferatum* ) وتأثير اثنين من المبيدات الفطرية (Hymexazol و Benomyl) تجاه نفس الفطريات. أظهرت النتائج وجود تثبيط كامل للنمو الشعاعي للفطرين *P. expansum* و*F. Proliferatum* عند التركيز 4.5 و 5%على التوالي للزيت الطيار لنبات الليمون 5.5 و 6% على التوالي للزيت الطيار للالنكي. كذلك تثبيط النمو الشعاعي للفطرين من قبل اثنين من المبيدات الفطرية، في حين أظهرت التركيزات الادنى زيادة في معدل التثبيط مع زيادة التركيز لكل المواد المستعملة بالدراسة سواء كانت زيت طيار او مبيدات.

**كلمات مفتاحية:** زيوت أساسية، قشور الحمضيات، الفطريات.

## Introduction

Mandarin and lemon belong to family Rutaceae and order Geraniales which return to sub-class Archichlamyae (1). The orginsim area lemon (*Citrus limon*) is still uncertain. It must have originated somewhere in southeastern Asia while China is one of the native homes of the mandarin (*Citrus reticulata*) (2). Essential oils are complex natural mixtures of volatile secondary metabolites, isolated from plants by hydro- or steam distillation and by expression (3). The main constituents of essential oils are monoterpens and sesquiterpenes including carbohydrates, alcohols, ethers, aldehydes and ketones which are responsible for the fragrant and biological properties of aromatic and medicinal plants (4). Citrus essential oils are present in fruit flavedo in high quantities. Peels consists of the epidermis covering the exocarp consisting of irregular parenchymatous cells, which are completely enclosing numerous glands or oil sacs. Citrus essential oils are a mixture of volatile compounds and which mainly consisted of monoterpene hydrocarbons. The terpene fraction can be constitute from 50 to more than 95% of the oil; however, it makes little contribution to the flavor and fragrance of the oil (5). Limonenes are found in the essential oil of various citrus leaves and fruit peels and have inhibited properties of both insects and fungi (6). Many studies investigated the essential oils of *Citrus limon* and *Citrus reticulate* against the growth of fungi (7,8,9,10,11,12,13,14,15,16,17).

The high quantity of citrus peel found as industrial waste and citrus industry presents a potential pollution problem, which would be reduced if the waste could be utilized as food of animals (18). Thus the aim of this study uses this waste as fungicide against some fungi and evaluates the effect of fungicides on the growth of fungi and this study is novel in Iraq.

## Material and Methods

**Plant collected**: The samples of *Citrus lemon* and *Citrus reteculata* collected in March 2012 from local market after the fruits had been washed, they were cut into six equal portions and the flesh was removed and used directly without dried (15)

**Fungi**: *Penicillium expansum* isolate was obtained from University of Baghdad, College of Science, Biology Department, while fungi isolate *Fusarium proliferatum* was obtained from fungi Laboratory postgraduate in University of Baghdad, College of Education Pure Science, Ibn al-Haitham, Biology Department and re-diagnosed and confirmed for them.

**Fugicides:** Pesticides Hymexazol and Benomyl (benzimidazole) have been obtained from the local markets and prepared according to the described method.

**Extraction of essential oils by steam distillation:** The extraction of essential oils have been extracting according to (12) protocol briefly, a 100 gm from fresh peels of plant and placed in the round bottom flask and filled with 1000 ml of distilled water then distillation apparatus was connected to the flask and take at 60 C. Distillation was continued until there was no more difference in successive readings of the oil volume. The yield of essential oil (%) was calculated as follows (16):

$$Yield\ of\ essential\ oil\% = \frac{Volume\ of\ essential\ oil\ (mL)}{Fruit\ peel\ sample\ (g)}\ x\ 100$$

Oils collected in distilled dark bottles until used.

**Test of sensitive of fungi against essential oils**

Each of Petri dish contained potato dextrose agar (PDA) and prepared the concentration below:

*C. limon* against *P. expansum* (1, 2, 3, 4, 4.5) (v/v)%

*C. limon* against *F. proliferatum* (1, 2, 3, 4, 4.5, 5) (v/v)%
*C. reteculata* against *P. expansum* (1, 2, 3, 4, 5, 5.5) (v/v)%
*C. reteculata* against *F. proliferatum* (1, 2, 3, 4, 5, 5.5, 6) (v/v)%
Dishes were inoculated with the fungus by cutting a 4 mm-diameter disc from pure cultures of *P. expansum* and *F. proliferatum* growing on PDA using a cork borer. This was done for each of concentration as well as for control (without essential oil).The cultures were incubated at 27 C in incubators for 6 days. Radial growth rate and inhibition ratio as estimated by measuring the maximum diameter of colonies was measured after 3-6 day and the ratio diameter/time was calculated by used the formula as shown below:

$$\% \text{ Growth inhibition} = \frac{DC - DT}{DC} \times 100 \qquad (2)$$

DC = The diameter of mold colony from control plate.
DT = The diameter of the mold colony growth in experiment plate which contains the essential oil (9).

**Test of sensitive of fungi against fungicide**

By using the method of (18), the radial growth rate and inhibition ratio was measured according to formula (2).The concentrations of fungicides were prepared:
Hymexazol against *P. expansum* and *F. proliferatum* (50, 100, 150, 200, 225) (w/v)% from the stock solution 36%.
Benomyl against *P. expansum* and *F. proliferatum* (50, 100, 150, 200, 225) (w/v)% from the stock solution 50%.

**Statistical Analysis**

All determinations were made in triplicates and the data is reported as mean ± SE for (n = 3). Analysis of Variance (ANOVA) method was used for statistical analysis and at probability levels (0.05, 0.01,0.001) for the purpose of evaluating the differences in the results of transactions in terms of being significant (influence of material) or not significant differences (as a result of laboratory errors) (19) .

**Results and Discussion**

**-Essential oils:** The results had been show that for every 100 grams of *C. limon* and *C. reteculata* peels contain (0.12, 0.08) ml [(0.12%, 0.08%) according to formula (1)] of essential oils respectively, this result is consistent with (20) they found that every 100 grams of *C. reteculata* peels contain 0.089 essential oils extracted by steam distillation.

**-Sensitive of fungi against essential oils:** The results showed that the treatment of fungi, *P. expansum* and *F. proliferatum* with essential oil extracted from the peel of fruits, *C. lemon* and *C. reteculata* have antifungal activity and gradually influenced the growth of these two isolates by increasing low concentration killer below the level of probability (0.001, 0.05, 0.01).The sensitivity of fungi were differed from two essential oil (Figure 1, 2, 3 and 4), where the complete inhibitory effect of essential oil of *C. lemon* on *P. expansum* and *F. proliferatum* were (4 and 4.5)% respectively, and for *C. reteculata* were (5.5 and 6)% . This results are agreed with (7) and (11) which found the essential oils of *C. lemon* and *C. reteculata* have effective toward *Penicillum* sp. and agreement with (8) that found there was an inhibitory of the essential oil *C. lemon* on *Fusarium* spp. and *Penicillium* spp. grown while the recent study was disagreed with studies (9) and (13) those found concentration 10% from *C. lemon* inhibited growth of *Fusarium* spp., moreovere, the activity of fungicidal against the myceliae growth of *Fusarium* spp. was observed at a concentration of 4% of lemon essential oils in study (10), which is consistent nearly with what was found in this study (figure 2). Study (14) found that the essential oil of lemon extract has inhibition effect toward four food-borne fungal strains including *Fusarium* spp. The results showed also that the essential oils of *C. lemon* has a significant effect on *Penicillum* sp. growth

than *Fusarium* spp. at below the level of probability (0.001, 0.05, 0.01) (Figure 1,2). These findings are agreement with (11) that concluded hydrodistillated-essential oils from all citrus cultivars were strong antifungal agents toward *Penicillium* spp. While the essential oil of *C. lemon* showed inhibitory action more than essential oil of *C. reteculata* at below the level of probability (0.001, 0.05, 0.01) (figure 1,2,3 and 4), this agreement with (7) which is found that the essential oil of *C. lemon* have more inhibitory action against *Penicillum* spp. than *C. reteculata*. The essential oil of *C. reteculata* significantly inhibited the growth of *Penicillum* spp. more than *Fusarium* spp. at below the level of probability (0.001, 0.05, 0.01) (figure 3,4), this found agreed with (14) which reached that *Penicillum* spp. more affected than other fungi including *Fusarium* spp. and the essential oil extracted from *C. reteculata* has inhibition activity and agreement with (17) that found the essential oil of *C. reteculata* has effective inhibition toward *F. proliferatum*, but less than the impact of essential oil of *Citrus aurantifolia*.

This inhibitory action can be explained by the demonstration of the action of essential oils on the wall of fungi whose structure and function are altered and the transport of nutrients is modified (9). Essential oils extracted from aromatic plants such as *C. lemon* and *C. reteculata* that possess antimicrobial activity, while diameters of treated colonies were smaller than control group, depending on the concentration of oil (14). Extracts of citrus plants contain antifungal compounds that can be used as alternative to synthetic fungicides including fumigants and contact pesticides. The prospect of using citrus for development of natural fungicides is appealing and acceptable because citrus peels are readily available, environmentally safe, and less risky for developing resistance in pets, less hazardous to non target organisms and pest resurgence, less adverse effect on plant growth, less harmful to seed viability and quality and above all less expensive. Based on these findings, citrus plant extracts are viable and can be possible alternative to synthetic pesticides for control of fungal diseases [9].

In a number of citrus species, the bitterness causative factors are limonoids, limonine being one of the potential antipest compounds known. A few other citrus limonoids including nomiline, nomilinic acid, ichangin and obacunoic acid are also bitter. Among these, limonine and nomiline are known to deter feeding in lepidopterans and coleopterans with variable efficacies. It appears that furan and epoxide groups play an important role in the activity of these compounds (21). Tangerine oil was obtained from the peel of *Citrus reticulate* is pleasant in taste and rich in aroma and is mainly used in food and beverages as flavoring agent.

As the essential oil is rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, that are found to have effective as antimicrobial properties. The monoterpenes affect the structural and functional properties of lipid fraction of the plasma membranes of bacteria and yeasts, causing leakage of intercellular material and exit of critical molecules and ions leading to death of microbes. Terpenoids affect respiratory enzymes inhibiting microbial oxygen uptake and oxidative phosphorylation (12).

**-sensitive of fungi against fungicide**

The results had been shown in figure 5, 6, 7 and 8 suggested that there is a significant effectiveness of the fungicides used against two fungal isolates (*P. expansum* and *F. proliferatum*) at below the level of probability (0.001, 0.05, 0.01) and fungicide hymexazol more effective on two fungus than the fungicide Benomyl (Figure 5, 6, 7, 8) while the fungus *P. expansum* was more influenced by both fungicides (Figure 5,7) at below the level of probability (0.001, 0.05, 0.01). Benomyl used against *Fusaium* sp.**,** when infected

guava wilt disease where added at concentration 0.2% (22) and inhibited the growth of *Fusarium* sp. by 83.64% (9), as also hymexazol used against *Fusarium* sp. (23). Study (24) was concluded that hymexazol at the dose recommended by the manufacturer significantly reduced disease incidence for a period of 2 months after transplanting. However Benomyl is unstable and easily decomposed to methyl 2-benzimidazole carbamate in aqueous solutions (25).

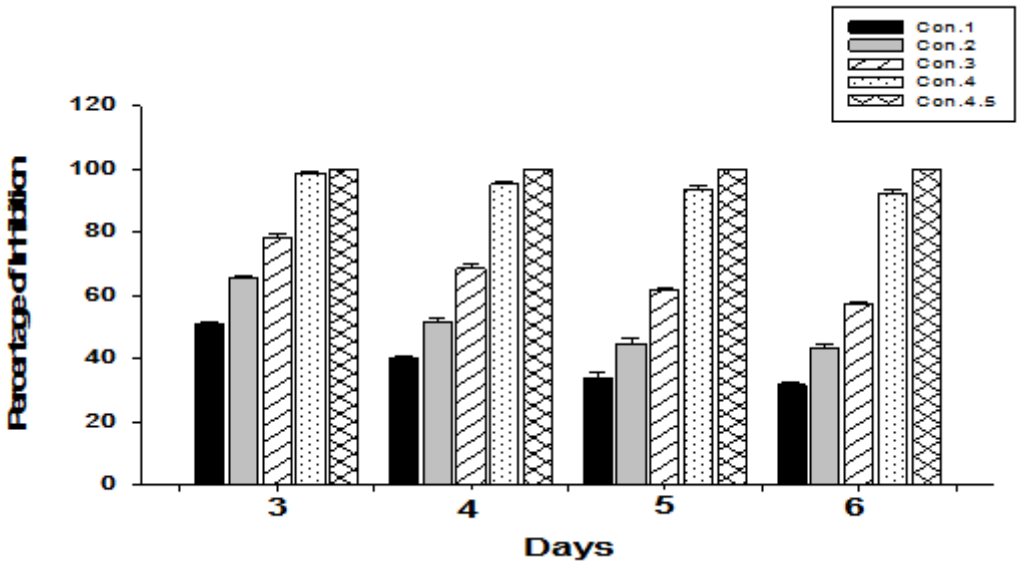


Figure (1): Inhibition% for different concentrations of essential oil of peel fruits of *C. limon* in surface growth of the fungus *P. expansum*.
Less significant difference (LSD) at 0.05 level for days 1.06 for concentrations 1.05 and interference 1.74.

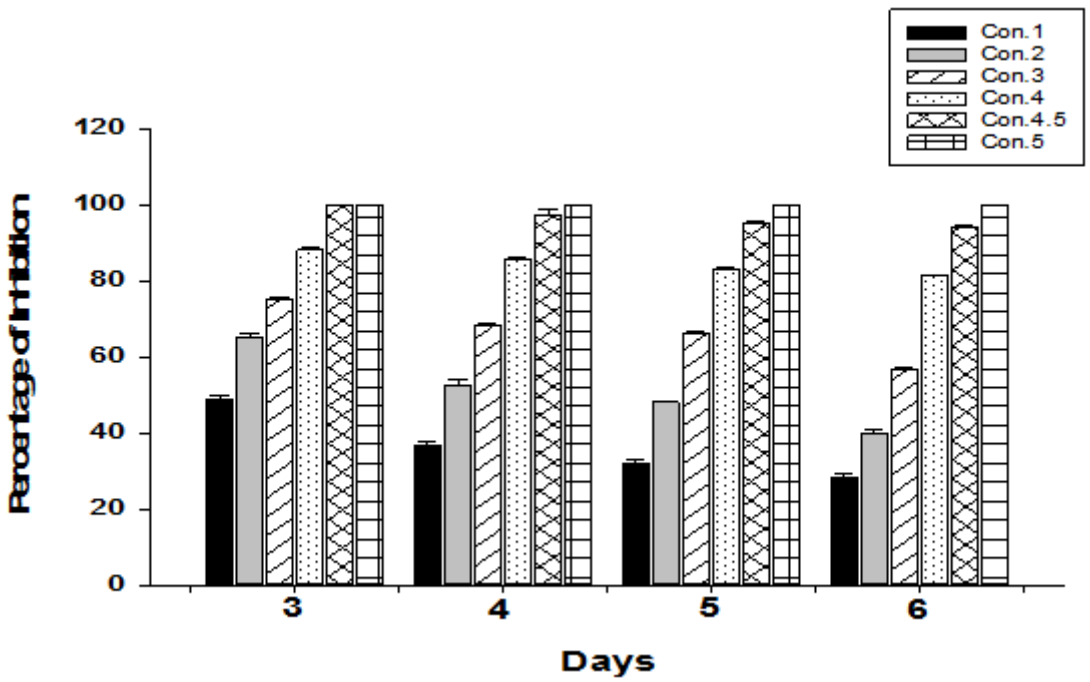


Figure (2): Inhibition% for different concentrations of essential oil of peel fruits of *C. limon* in surface growth of the fungus *F. proliferatum*.

Less significant difference (LSD) at 0.05 level for days 1.33 for concentrations 1.00 and interference 1.72.

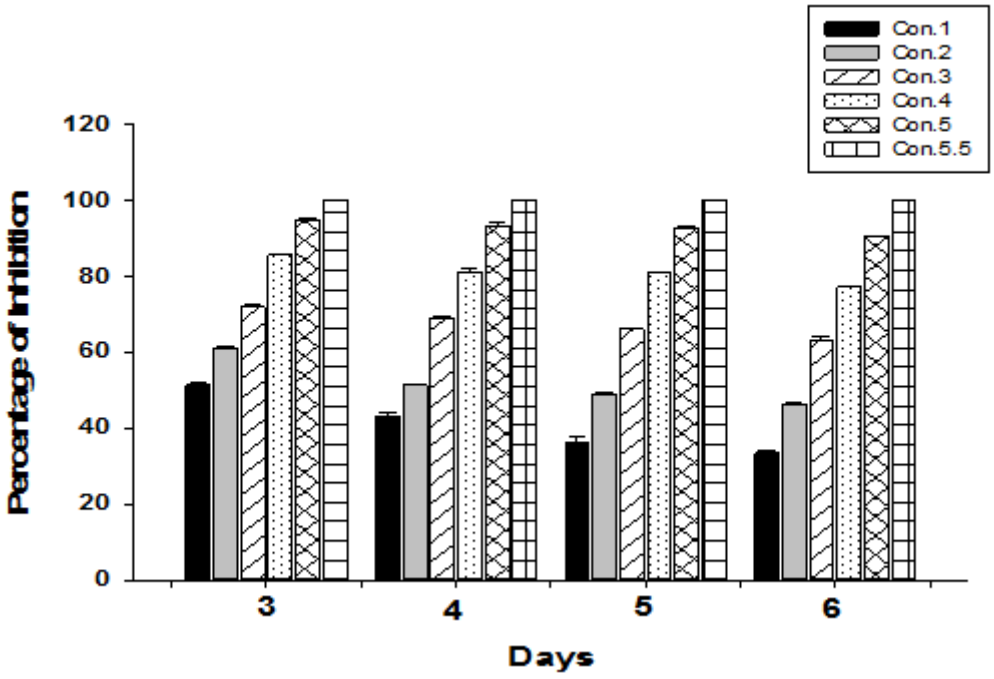


Figure (3): Inhibition% for different concentrations of essential oil of peel fruits of *C. reticulata* in surface growth of the fungus *F. expansum*.
Less significant difference (LSD) at 0.05 level for days 1.01 for concentrations 0.76 and interference 1.31.

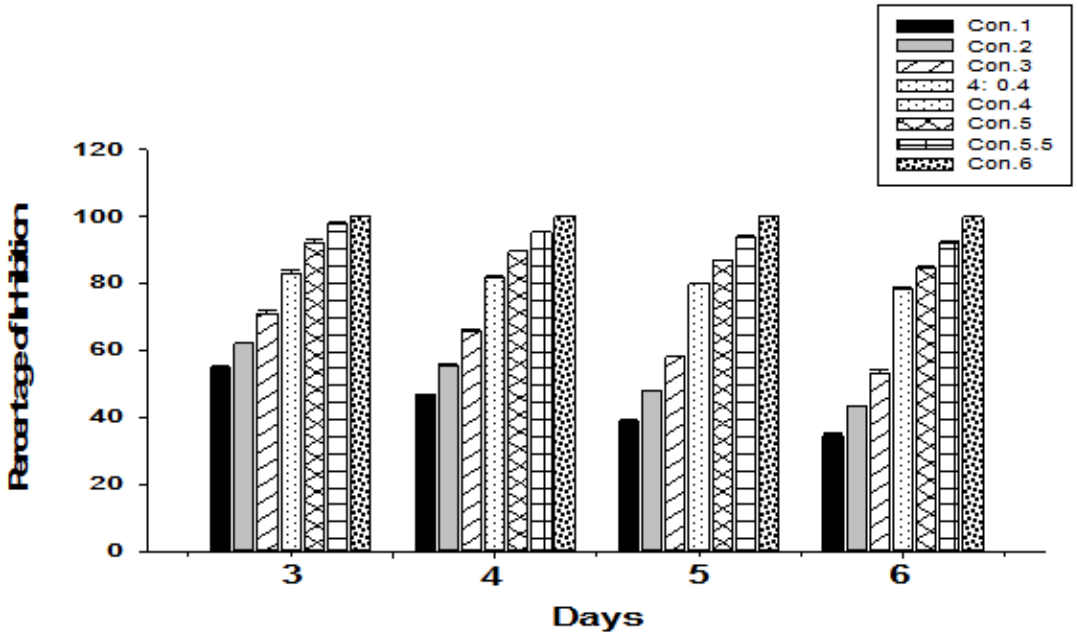


Figure (3): Inhibition% for different concentrations of essential oil of peel fruits of *C. reticulata* in surface growth of the fungus *F. proliferatum*. Less significant difference (LSD) at 0.05 level for days 2.68 for concentrations 2.02 and interference 3.47.

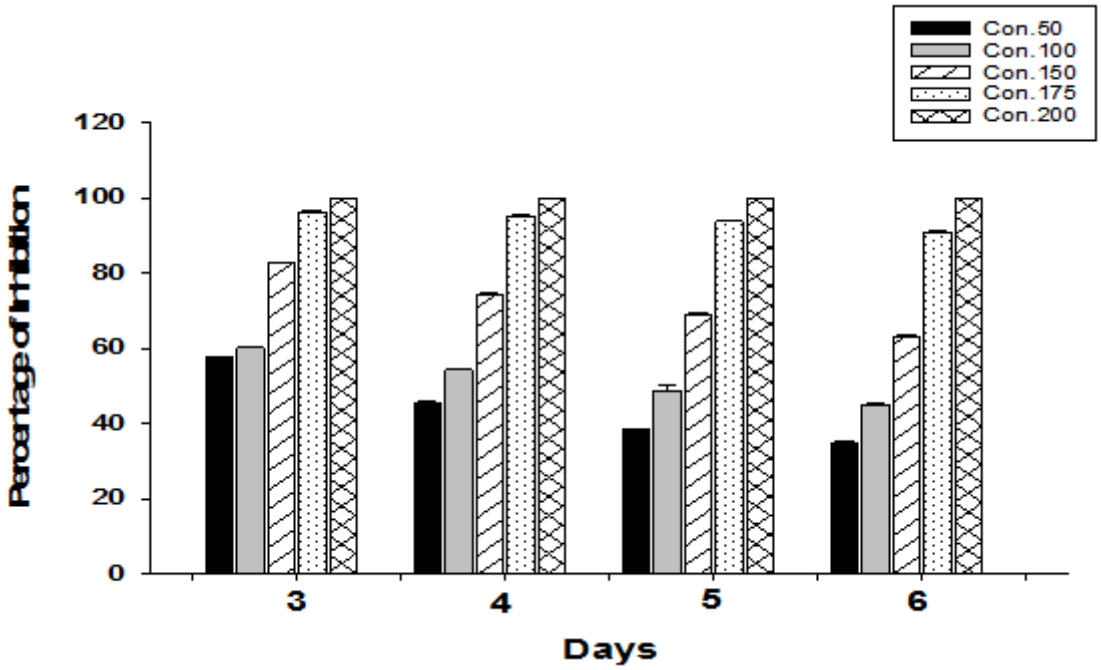


Figure (5): Inhibition% for different concentrations of Hymexazol in surface growth of the fungus *P. expansum*.

Less significant difference (LSD) at 0.05 level for days 1.33 for concentrations 1.32 and interference 2.19.

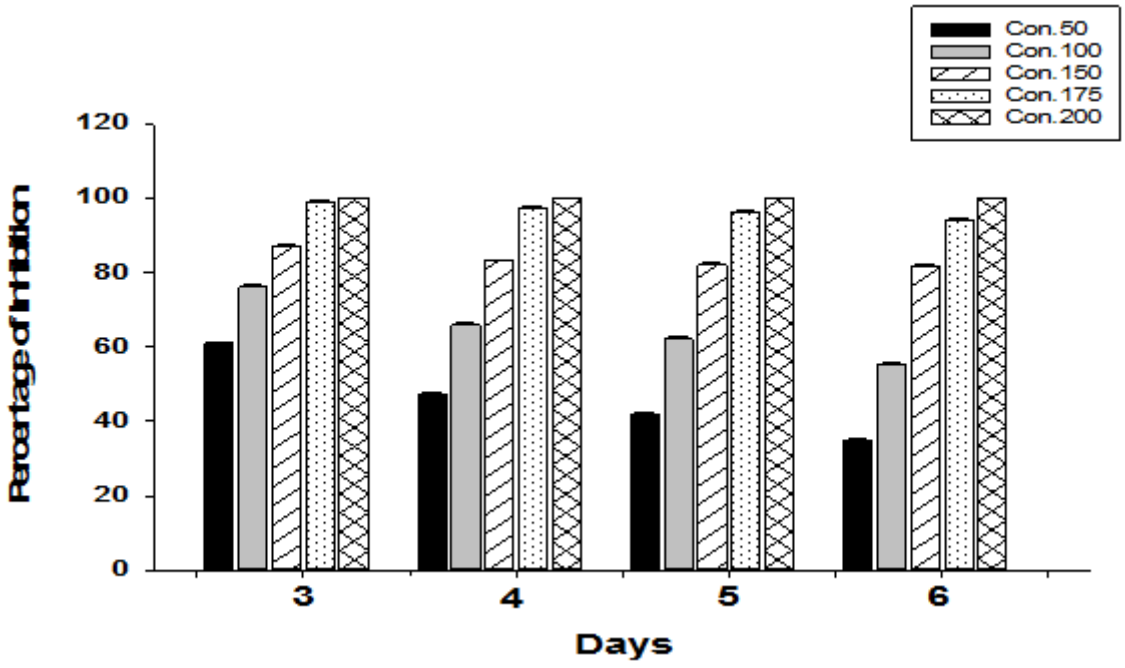


Figure (6): Inhibition% for different concentrations of Hymexazol in surface growth of the fungus *F. proliferatum*.

Less significant difference (LSD) at 0.05 level for days 1.62 for concentrations 1.60 and interference 2.66.

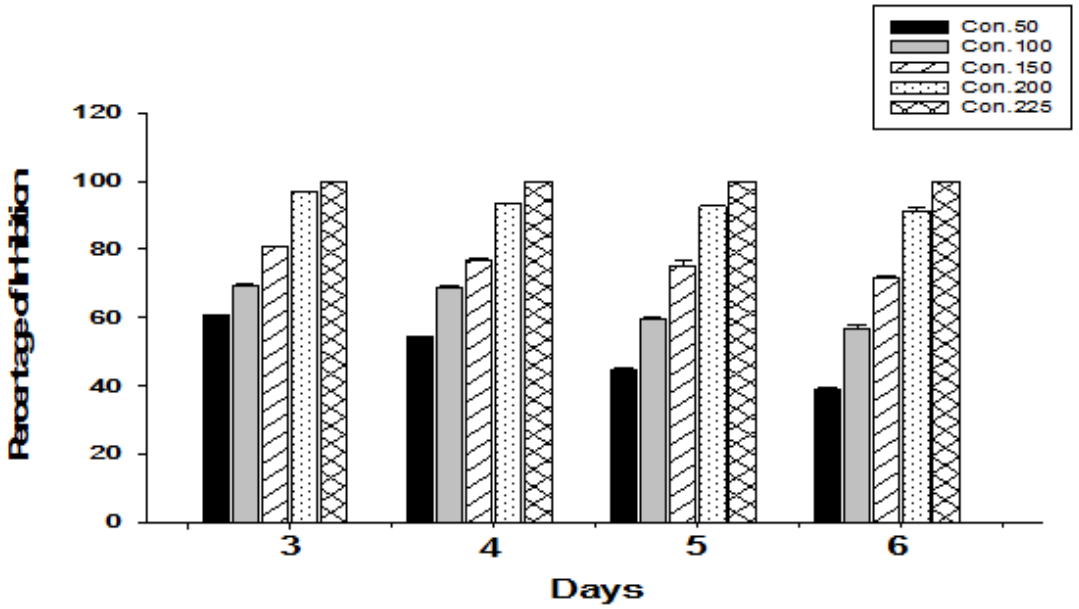


Figure (7): Inhibition% for different concentrations of Benomyl in surface growth of the fungus *P. expansum*.

Less significant difference (LSD) at 0.05 level for days 1.99 for concentrations 1.97 and interference 3.26.

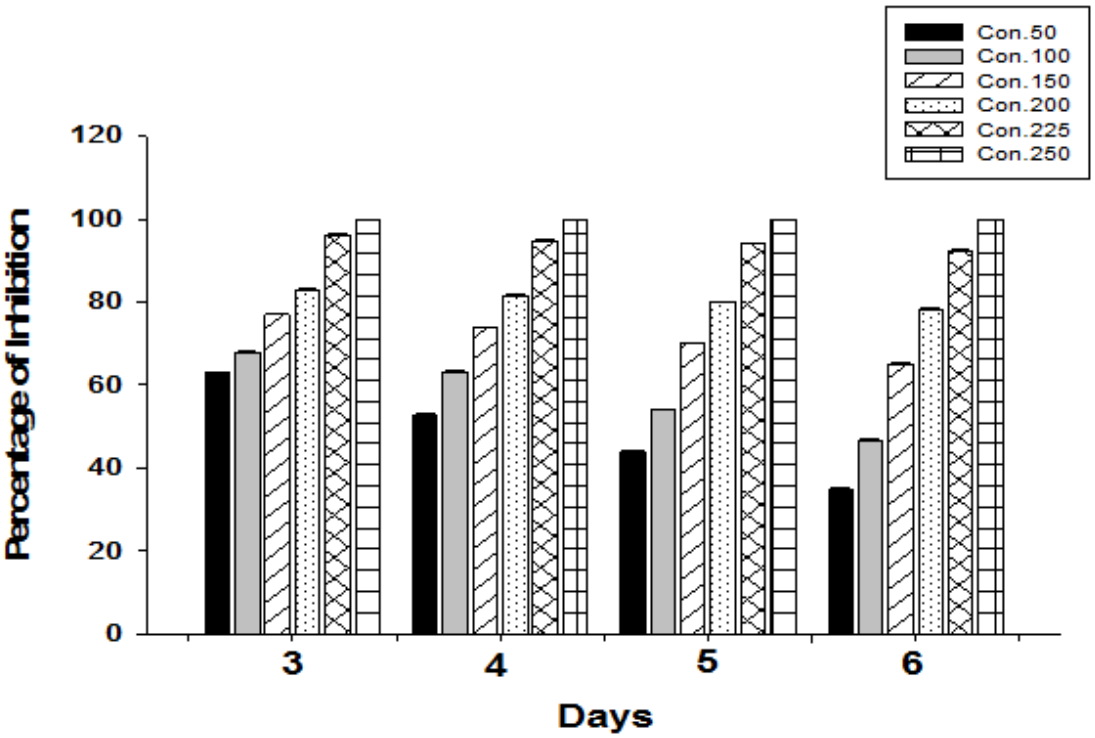


Figure (8): Inhibition% for different concentrations of Benomyl in surface growth of the fungus *F. proliferatum*.

Less significant difference (LSD) at 0.05 level for days 2.03 for concentrations 1.53 and interference 2.62.

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