(IJRST) 2015, Vol. No. 5, Issue No. III, Jul-Sep **e-ISSN: 2249-0604; p-ISSN:2454-180X**

EFFECT OF ESSENTIAL OIL EXTRACTED FROM THE YELLOW PEEL OF *CITIRUS AURANTIUM* ON SOME FUNGI

***Hayfaa A. Yousif**
****Estabraq A. Mahmoud**
**Department of Biology*
College of Education for Pure Science/ Ibn-Al-Haitham, University of Baghdad
Baghdad, Iraq.
***Department of Biology*
College of Education for Pure Science/ Ibn-Al-Haitham, University of Baghdad
Baghdad, Iraq.

ABSTRACT

This study was aimed to investigate the effect of essential oil extracted from the yellow peels of Citrus aurantium on the growth of four species of fungi: Penicillium expansum, Penicillium oxalicum, Fusarium oxysporum and Fusarium proliferatum and effect of one fungicide: Aliette (fosetyl-aluminum) against these fungi. The results showed that the essential oil of C. aurantium inhibited the radial growth of P. oxalicum at concentration 4.5% while P. expansum and F. oxysporum at concentrations 5% and F. proliferatum at concentrations 5.5% additionally the one fungicide tested showed inhibitory effect on radial growth of these fungi. So that there is a negative relationship between the increasing of concentration and radial growth of fungi.

Keywords: *essential oil; C. aurantium; Antifungal; Penicillium spp.; Fusarium spp.*

1. INTRODUCTION

Citrus aurantium (Bitter orange) belong to family Rutaceae and order Geraniales which return to sub-class Archichlamyae [1]. The original area is southern Europe and other subtropical areas, particularly Spain, Portugal, and the various islands of the Caribbean [2]. *C. aurantium* peel contains a essential oil with limonene (about 90%), flavonoids, coumarins, triterpenes, vitamin C, carotene, and pectin [3]. Essential oils are complex natural mixtures of volatile secondary metabolites, isolated from plants by hydro- or steam distillation and by expression [4]. 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 [5]. 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 [6]. Limonenes are found in the essential oil of various citrus leaves and fruit peels and have inhibited properties of both insects and fungi [7]. Many studies investigated the essential oils of *Citrus aurantium* against the growth of fungi [8, 9, 10, 11].

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 [12]. The aim of present work is the evaluation of inhibitory effect of essential oil of peels for citrus fruits against four plant pathogenic fungi compared with Aliette who is a trusted and well-known Highly Systemic fungicide.

2. MATERIALS AND METHODS

Plant collected: The fruits of *Citrus aurantium* was purchases in March 2014 from local market. After the fruits had been washed, they were cut into six equal portions and the peels were removed and used directly without drying [13].

2.1 Fungi: *Penicillium expansum* isolate was obtained from University of Baghdad/ College of Science / Biology Department, while *P. oxalicum*, *Fusarium oxysporum* and *Fusarium proliferatum* were obtained from postgraduate Mycology lab University of Baghdad/ College of Education Ibn al-Haitham/ Biology Department. These two isolates were re-identified according to [14].

2.2 Fungicide: Pesticide Aliette (fosetyl-aluminum) has been obtained from the local markets and prepared according to the described method in [15] and [16].

2.3 Extraction of essential oils by steam distillation: The essential oil extractions have been performed according to [12] protocol. In brief, a 100 gm of fresh peels added to with 1 liter of distilled water in the flask of the equipment at 60 C for 6 hours. The essential oil eluted and the percentage of concentration calculated as follow [11]:

$$\text{Yield of essential oil (\%)} = \frac{\text{Volume of essential oil (mL)}}{\text{Fruit peel sample (g)}} \times 100 \quad (1)$$

Oils were preserved in sterile dark bottles until used.

2.4 Sensitivity test of fungi against essential oils

Each essential oil was added to the PDA medium separately at final concentration of (1-6)% according to [15]. Petri dishes were inoculated with the fungus by cutting a 4 mm-diameter disc from pure cultures of *P. expansum, P. oxalicum, F. oxysporum* 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 for 3-6 days. Radial mycelium growth rate and inhibition ratio as estimated by measuring the diameter of colonies was measured after 3-6 days and inhibition ratio was calculated according to the following equation [17]:

Inhibition (%) = {1 - [radial growth of treatment (mm)/radial growth of control (mm)]}×100 (2)

2.5 Sensitivity test of fungi against fungicide

In the same way each pesticide was added to the PDA medium separately at final concentration (50-275) (w/v)% from the stock solution Aliette 80W [15]. The radial mycelium growth and inhibition ratio calculated according to above equation described by [17].

2.6 Statistical Analysis

All determinations were made in triplicates and the data reported as mean ± SE for (n = 3). Analysis of Variance (ANOVA) method used for statistical analysis and at $P< 0.05, 0.01, 0.001$ 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) [18].

3. RESULTES AND DISCUSSION

3.1 Essential oils: Results of extracting essential oil from *C. aurantium* showed clear yellow essential oil with a fresh sweet odor was obtained through the steam distillation of peel at 1.47% (mL/100 g of fresh tissue). This result is consistent with [19] who found amount of 1.67% essential oil from *C. aurantium* peels extracted by steam distillation and peel oil is composed almost of monoterpene hydrocarbons (98.30%), mainly limonene.

3.2 Sensitivity test of fungi against essential oils: Treatment of *P. expansum, P. oxalicum, F. oxysporum* and *F. proliferatum* with essential oils of *C. aurantium* yellow peel showed inhibitory activity toward the four fungi and this inhibition increased with increasing concentration (Figure 1,2,3 and 4), it is also notable that growth inhibition percentage was increased with incubation time. This observation was also shown by many researchers who reported that the degree of fungal growth inhibition was increased with incubation time [20, 21].

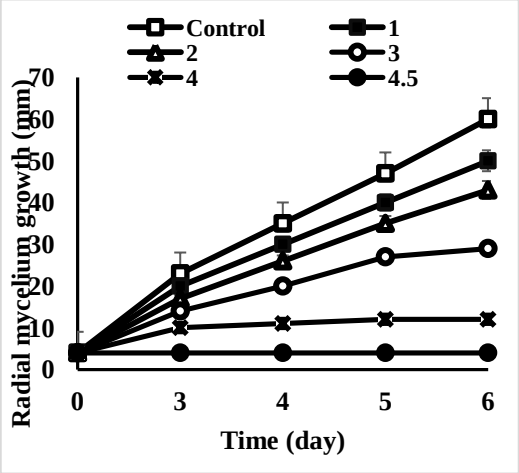

Figure (1): Effect of essential oil of C. aurantium yellow peel on radial mycelial growth of the fungus P. oxalicum (Significant at P<0.01).

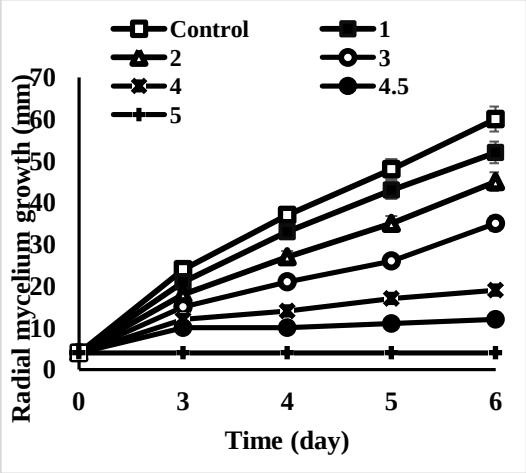

Figure (2): Effect of essential oil of C. aurantium yellow peel on radial mycelial growth of the fungus P. expansum (Significant at P<0.01)

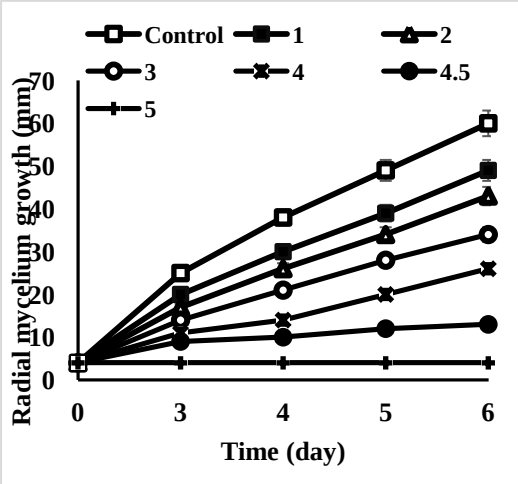

Figure (3): Effect of essential oil of C. aurantium yellow peel on radial mycelial growth of the fungus F. oxysporum (Significant at P<0.05).

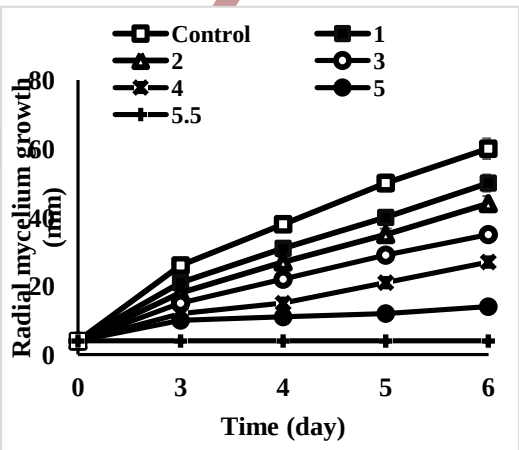

Figure (4): Effect of essential oil of C. aurantium yellow peel on radial mycelial growth of the fungus F. proliferatum (Significant at P<0.01).

Moreover sensitivity of fungi was varied with of essential oils of *C. aurantium* and the complete inhibitory effect have been shown with the essential oil of *C. aurantium* on *P. oxilicum* and *P. expansum* (4, 4.5)% respectively. These results agreed with [8] and [10] who found that the essential oil of *C. aurantium* were effective on *Penicillum* sp. growth and also this study consisted with [15] who found inhibitory effect of the essential oil from *C. aurantium* peel against growth of three species from *Penicillium* spp. that were *Penicillium digitatum*; *Penicillium italicum* and *Penicillium expansum*, and last one was the most affected.

The essential oil from *C. aurantium* inhibited growth of *F. oxysporum* but lesser than *P. oxilicum* these finding agreed with [11] who found antifungal activity of *C. aurantium* flowers against growth of 8 species of fungus included *F. oxysporum*.

The results of this study also showed significant inhibitory effect of *C. aurantium* essential oil on *P. oxilicum* and *P. expansum* growth compared to *F. oxysporum* and *F. proliferatum* (Figure 1,2,3 and 4) and this was agreed with [22] who concluded that the essential oil of all *Citrus* sp. have significant inhibitory effect toward *Penicillium* spp. growth.

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 [15]. The high antimicrobial activity of these essential oils could be explained by their high percentage of phenol components. It seems likely, that carvacrol interferes with the activity of cell wall enzymes like chitin synthase/chitinase as well as α- and β- glucanases of fungi [23].

As the essential oil of citrus peels is rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, which 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].

Extracts of citrus plants contain antifungal compounds 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 [15].

4.3 Sensitivity test of fungi against fungicide

Treating four fungi with fungicide Aliette, results showed significant effect of both fungicides toward the four fungi (Figure 5, 6, 7 and 8) (P<0.001, 0.005, 0.01), although, the fungus *P. oxilicum* showed more sensitivity against fungicide compared with other fungi used in these study (Figure 5, 6, 7 and 8). These results coincided with [24] who confirmed the effect of Aliette on *F. oxysporum* and other 38 species of fungi, [25] were concluded that Aliette (fosetyl -Al) has been found to be an effective fungicide in reducing losses in young citrus trees to *Phytophthora* spp. As well [26] used it as fungicide for control of *Alternaria* brown spot and citrus scab and noted only a few fungicide such as Aliette (fosetyl -Al) proved to be infective against scab.

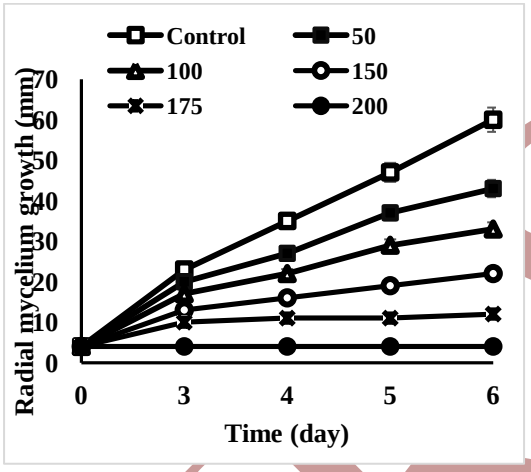

Figure (5): Effect of Aliette on radial mycelial growth of the fungus P. oxalicum (P< Significant at 0.01).

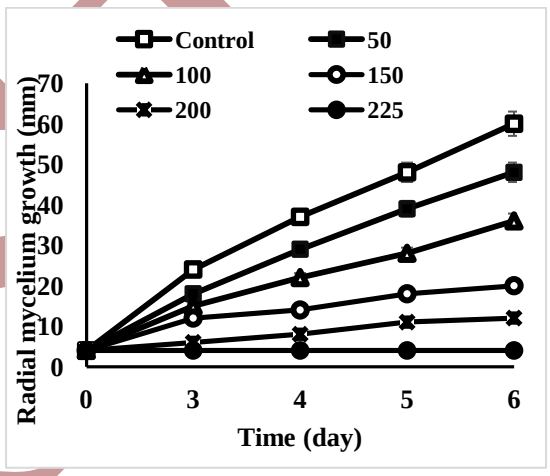

Figure (6): Effect of Aliette on radial mycelial growth of the fungus P. expansum (P< Significant at 0.05).

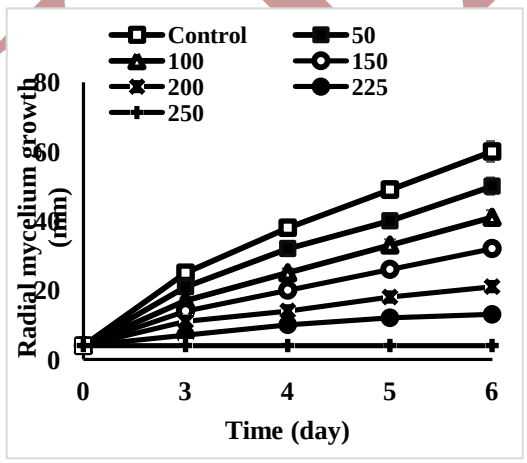

Figure (7): Effect of Aliette on radial mycelial growth of the fungus F. oxysporum (Significant at P<0.05).

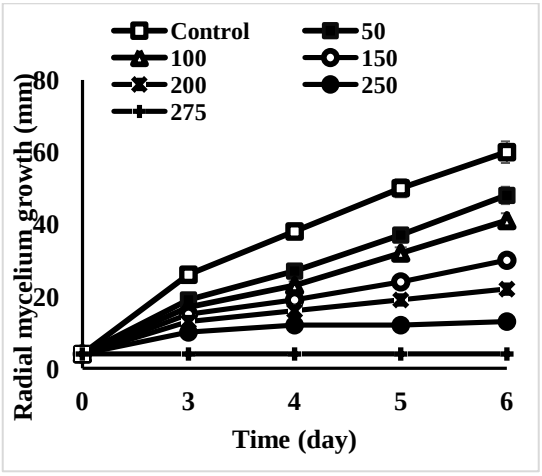

Figure (8): Effect of Aliette on radial mycelial growth of the fungus F. proliferatum (Significant at P<0.01).

5. REFERENCES

[1] B. Bhattacharyya, and M. M. Johri, "Flowering plant taxonomy and phylogeny," Navosa publishing house, New Delhi, pp 753, 1998.

[2] M. Blumenthal, "Bitter Orange Peel and Synephrine: Part 1.," Reprinted with permission from Whole Foods March 2004 issue, 2004.

[3] N. Khazaei, M. Jouki, and A. Jouki, "Effects of Extended Storage on Bitter Orange Quality Factors," Res. J. Appl. Sci. Eng. Technol., vol. 4, No15, pp. 2296-2299, 2012.

[4] P. Tripathi, and A. K. Shukla, "Exploitation of botanicals in the management of phytopathogenic and storage fungi," In: A. Arya, and A. E. Perelló, (Eds.), "Management of fungal plant pathogens," MPG books Group, UK pp. 388, 2010.

[5] D. Kalemba, and A. Kunicka, "Antibacterial and antifungal properties of essential oils," Curr. Med. Chem., vol. 10, pp. 831-829, 2003

[6] S. Chanthaphon1, S. Chanthachum, and T. Hongpattarakere, "Antimicrobial activities of essential oils and crude extracts from tropical *Citrus* spp. against food-related microorganisms," Songklanakarin J. Sci. Technol., vol. 30(Suppl.1), pp. 125-131, 2008.

[7] P. Koul, S. Walia, and G. S. Dhaliwal, (2008). "Essential Oils as Green Pesticides: Potential and Constraints," Biopestic. Internat., vol. 4, No, pp. 63-84, 2008

[8] D. R. L. Caccionia, M. Guizzardia, D. M. Biondib, A. Rendab, G. Rubertob, "Relationship between volatile components of citrus fruit essential oils and antimicrobial action on *Penicillium digitatum* and *Penicillium italicum*," Int. J. Food Microbiol., vol. 43, No 1–2, pp. 73–79, 1998.

[9] R. R. Stangea, S. L. Midlandb, J. J. Simsb, and T. G. McColluma, "Differential effects of citrus peel extracts on growth of *Penicillium digitatum*, *P. italicum*, and *P. expansum*," Physiol. and Molecul. Plant Pathol., vol. 61, No 5, pp. 303–311, 2002.

[10] D. J. Hall, and Y. J. Fernandez, "*In vitro* evaluation of selected essential oils as fungicides against *Penicillium digitatum sacc*," Proc. Fla. State Hort. Soc, vol. 117, pp. 377-379, 2004.

[11] A. Ben Hsouna, N. Hamdi, N. Ben Halima, and S. Abdelkafi, "Characterization of essential oil from *Citrus aurantium* L. flowers: antimicrobial and antioxidant activities," J. Oleo Sci., vol. 62, No 10, pp. 763-772, 2013.

[12] V. D. Zheljazkov, T. Astatkie, E. A. Jeliazkova, and V. Schlegel, "Distillation time alters essential oil yield, composition, and antioxidant activity of male *Juniperus scopulorum* trees," J. Oleo Sci., vol. 61, No. 10, pp. 537-546, 2012.

[13] F. G. Kirbaşlar, A. Tavman, B. Dülger and G. Türker "Antimicrobial activity of Turkish *Citrus* peel oils," Pak. J. Bot., vol. 41 no 6, pp. 3207-3212, 2009.

[14] J. I. Pitt, and A. D. Hocking, "Fungi and food spoilage," Springer Science + Business Media, LLC., London: pp. 519, 2009.

[15] D. E. Okwu, A. N. Awurum, and J. I. Okoronkwo, "Phytochemical composition and *in vitro* antifungal activity screening of extract from citrus plants against *Fusarium oxysporum* okra plant *Hibiscus esculentus*)," African Crop Conference Proceedings, vol. 8, pp. 1755-1785, 2007.

[16] B. V. Sagar, and P. K. Varma, "Practical manual of diseases of horticultural crops and their management," Acharya Ng Ranga Agricultural University, College of Agriculture, Rajendranagar-500030, pp. 72, 2012.

[17] M A. Numpaque, L. A. Oviedo, J. H. Gil1, C. M. García, and D. L. Durango, "Thymol and carvacrol: biotransformation and antifungal activity against the plant pathogenic fungi *Colletotrichum acutatum* and *Botryodiplodia theobromae*," Tropical Plant Pathology, vol. 36 No 1, pp. 3-13, 2011.

[18] J. H. Zar, "Biostatistical 4th. Prentice hall upper saddle river, analysis.New jersey, pp. 663, 1999.

[19] E. Sarrou, P. Chatzopoulou, K. Dimassi-Theriou, and I. Therios, "Volatile Constituents and Antioxidant Activity of Peel, Flowers and Leaf Oils of *Citrus aurantium* L. Growing in Greece," Molecules, vol. 18, pp. 10639-10647, 2013.

[20] S. Tharmila, E. C. Jeyaseelan, and A. C. Thavaranjit, "Preliminary screening of alternative culture media for the growth of some selected fungi," Arch. Appl. Sci. Res., vol. 3, No 3, pp. 389-393, 2011.

[21] A. Pereira, A. Santos, F. Reis, R. M. Tavares, P. Baptista, T. Lino-Neto, and C. Almeida-Aguiar, "A new effective assay to detect antimicrobial activity of filamentous fungi," Microbiol. Res., vol. 168, No 1, pp. 1-5, 2013.

[22] M. Viuda-Martos, Y. Ruiz-Navajas, J. Fernandez-Lopez, and J. Perez-Alvarez, "Antifungal activity of lemon (*Citrs lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradise* L.) and orange (*Citrus senisis* L.) essential oils," Food Control, vol. 19, No 12 pp.1130-1138, 2008.

[23] K. Adam, A. Sivropoulu, S. Kokkini, T. Lanaras, and M. Arsenakis, "Antifungal activities of *Origanum vulgare* subsp. hirtum, *Mentha spicata*, *Lavandula angustifolia* and *Salvia fruticose* essential oils against human pathogenic fungi," J. Agr. Food Chem., vol. 46, pp. 1739-1745, 1998.

[24] U. Sitara, and N. Hasan, "Studies on the efficacy of chemical and non chemical treatments to control mycoflora associated with Chilli seed," Pak. J. Bot., vol. 43 No 1 pp. 95-110, 2011.

[25] R. M. Sonoda, M. Vathakos, and R. R. Pelosi, "Interaction of fosetyl-aluminum fungicide and copper fungicides on citrus fruit and foliage," Proc. Fla. State Hort. Soc., vol. 102, pp. 10-13, 1989.

[26] L. W. Timmer, and S. E. Zitko "Evaluation of fungicide for control of Alternaria brown spot and citrus scab," Proc. Fla. State Hort. Soc., vol. 110, pp. 71-76, 1997.