Conference Proceedings of Science (CPS)
June, 2025
DOI: 10.69923/2detby27 Volume: 1 Issue: 1
18

Article

A Comparative Study of Penicillium italicum Filtrates Isolated from Rotted Orange

Anaam Fuad Hussain[1*] **Mohanad Waheeb Mahdi**[2]

Department of Biology, College of Science, University of Diyala, 32001 Diyala, Iraq
Department of Biology, College of Education for Pure Sciences, University of Diyala,32001, Ba'aqubah, Diyala, Iraq

ABSTRACT

Saprophytic species of *Penicillium* are among the best-known representatives of the Eurotiales and live mainly on organic biodegradable substances. The current study included *preparation of Penicillium italicum* filtrates which is the plant saprophyte that common in post-harvest disease commonly associated with citrus fruits. The objectives of our study were determination of the active medium for growing and production of *P. italicum* metabolites. Results revealed that the biomass of the mold growth was more weight in solid-state fermentation than in liquid fermentation. The crude extract of two replicates of *P. italicum* YES and CZ medium culture for the selective isolate active were 0.063 and 0.042 mg respectively. The crude extract of rotted orange with *P. italicum* was 0.11 mg.

Keywords *Penicillium italicum*, Fungi, Rotted Orange.

***Corresponding Author**

Anaam Fuad Hussain
Department of Biology, College of Science,,
University of Diyala, Baqubah City, Diyala Governorate,3200, Iraq.
Email: anaamfuad@Uodiyala.edu.iq

1. INTRODUCTION

Saprophytic species of *Penicillium* are among the best-known representatives of the Eurotiales and live mainly on organic biodegradable substances. The ability of these *Penicillium* species to grow on seeds and other stored foods depends on their propensity to thrive in low humidity and to colonize rapidly by aerial dispersion while the seeds are sufficiently moist (Mohammed *et al.*, 2018).

Penicillium italicum is a plant pathogen. It is a common post-harvest disease commonly associated with citrus fruits (Webster & Weber, 2007). In the agricultural sector, *Citrus* is one of the most important fruit genus in the world. The orange production has been considerable decreasing due to unfavorable weather conditions in recent years and the increasing number of pathogen infections. One of the main citrus post-harvest phytopathogen is *Penicillium italicum*, responsible for the blue mold disease (Kanashiro *et al.,* 2020).

Due to oranges acidic pH, around 4–5 in healthy fruits (Costa et al., 2019), most of the orange rot is caused by fungi and not bacteria. Phytopathogenic fungi can produce and proliferate mycotoxins, secondary metabolites of low molecular mass produced by filamentous fungi (Zain, 2011), which are often toxic to the host and other organisms that cohabit the same microenvironment.

Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes. Indexing and abstracting services depend on the accuracy of the title, extracting from it keywords useful in cross-referencing and computer searching.

Classification of *Penicillium italicum* according to Webster & weber, (2007)

Kingdom	Fungi
Division	Ascomycota
Class	Eurotiomycetes
Order	Eurotiales
Family	Trichocomaceae
Genus	*Penicillium*
Species	***P. italicum***

Binomial name		
Penicillium		*italicum*
Wehmer, (1894)		

The objectives of our study were determination of the active medium for growing and production of P. *italicum* metabolites.

2. Materials and Methods

Sampling and Identification of Penicillium italicum

A naturally rotten citrus fruit with Penicillium italicum was mixed with un infected samples at room temperature for two months (February and March)

The selective isolate of Penicillium italicum was isolated in Sabouraud Dextrose Agar for identification and then maintained in Potato Dextrose Agar slants and were placed in 4 °C as stock cultures (Oliver et al., 1982). Identification of the fungus was made using Lacto phenol cotton blue stain according to the bellow criteria:

1. Colony morphology (color and consistency)
2. Reverse color (color changed with age)
3. Microscopic characteristics (micro conidia and macro conidia, their size, arrangement, shape and conidial ontogeny).

Growth conditions of *Penicillium italicum* and determination of metabolites

The Spores of selected isolate was first ferment by growing on liquid culture of Yeast Extract Sucrose (YES) broth and Czapic Dox broth then incubate in state incubator for ten days at 22°C. Duplicate cultures of each media were inoculated with 1 ml of spore suspension.

The extraction was performed by adding 50 ml chloroform to 150 ml liquid culture in an Erlenmeyer flask after the infiltration of the culture. Products was separated from the liquid culture using separating funnel and evaporated to dryness in the open air by petri dishes at 45ºC , the extracts was preserved at dry and cold conditions at 4ºC (Mohammed,2018). The extraction from rotted orange (solid fermentation) was performed by adding 75 ml of chloroform to rotted fruit (one kilo of orange) with electric homogenizing with magnetic stirrer for 10 min. The extracted solution was then filtered through a Whatman No.1 filter paper and extracted using 50 ml of chloroform using separating funnel. The filtered, chloroform fractions were pooled and evaporated to dryness at 45°C. Dried extracts was stored at 4°C (Kosalec et al., 2005).

Determination the quantitations of *Penicillium italicum* filtrates

The filtrates after being collected and dried were weighed so as to compare among the type of extraction, then dissolved in 50% of Di methylesulfonamide (DMSO) to prepare the stock solution of the fungal extract

3. RESULTS AND DISCUSSION

The biomass of the mold growth was more weight in solid state fermentation than in liquid fermentation. The crude extract of two replicates of P. italicum YES and CZ medium culture for the selective isolate active were 0.063 and 0.042 mg respectively.

The crude extract of rotted orange with P. italicum was 0.11 mg.

Figure 1. Rotted orange *Citrus sinensis with P. italicum*

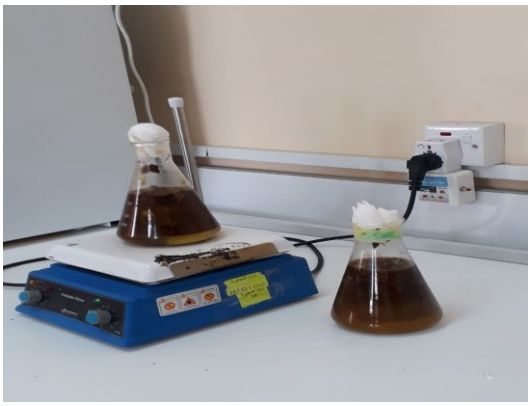

Figure 2. Electric Homogenizing of solid state fermentation culture of *P. italicum*

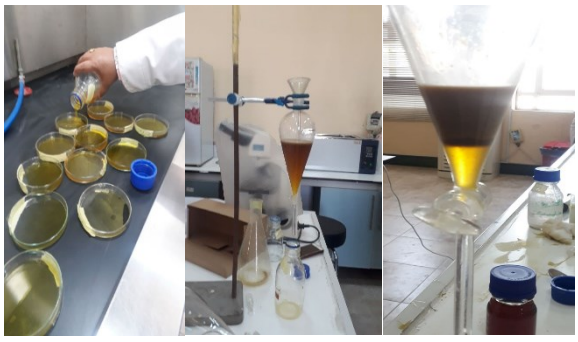

Figure 3. *Penicillium italicum* filtrate with chloroform

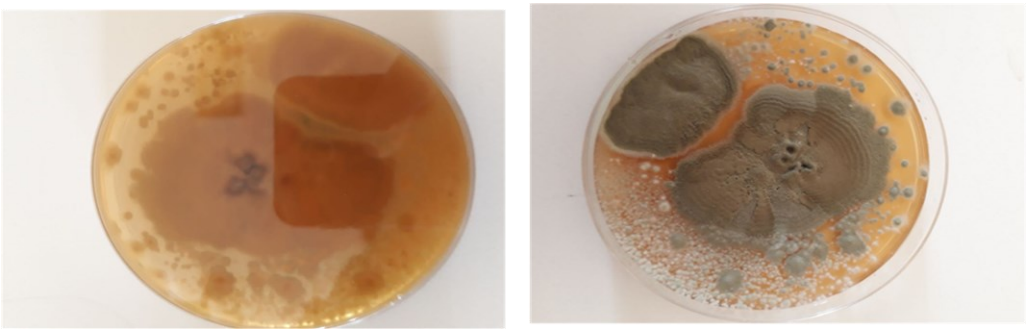

Figure 4: *P. italicum* on CZD Agar at 25℃ for 5 days

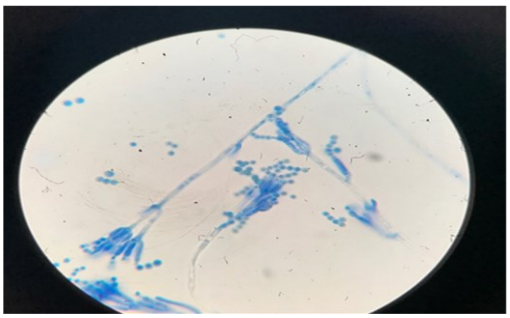

Figure 5: *P. italicum* stained with LPCB 40X

Figure 6: Rotted orange fruit

Figure 7: Preparation of liquid fermentation of *P.italicum*

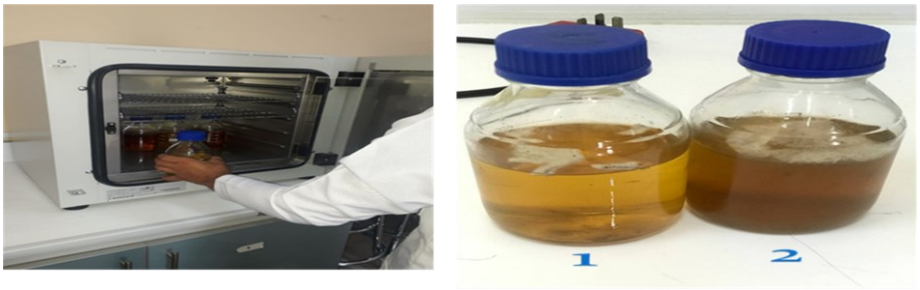

Figure 8: Liquid fermentation of *P.italicum* With state incubator 1. *Czapic medium* 2. *YES medium*

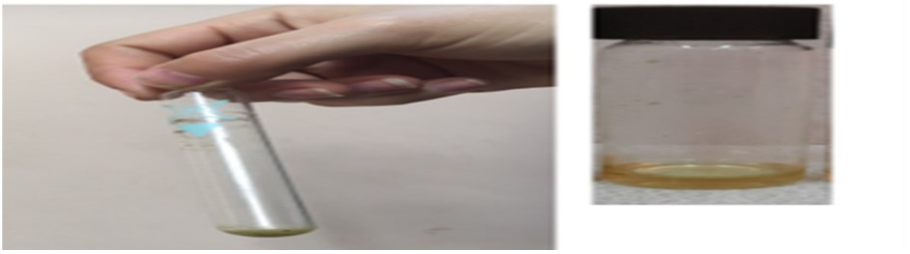

Figure 9: Dried filtrate of *P. italicum*

Figure 10: Stock filtrate dissolved in 50% DMSO

4. CONCLUSION

The crude extract from natural medium in solid state fermentation of rotted orange was more weighted than that was obtained from liquid fermentation of synthetic media. Use of natural media instead of artificial media for culturing Penicillium italicum,Study the comparative biological activities of P. italicum filtrates,Detect the chemical composition of these filtrate using chemical analysis methods such as GC-MS. Purification of the biological active compounds from these filtrates.

ACKNOWLEDGEMENTS

Praise is due to Allah, Lord of the whole creation, Mercy and Peace are to the Prophet Mohammad and his relatives and companions.

We would like to express our sincere thanks to our supervisor Dr. Anaam Fuad Hussain for her continuous support to finish this study.

It is a pleasure to thank all members of Biotechnology department at College of science of Diyala University for efforts all throughout the whole course of this work and for their help and support.

We also would like to express our deep indebtedness to our families, for supporting us in this work.

Finally, we are grateful to our faithful friends in our college and to whom that we didn't mentioned.

REFERENCES

[1] J. H. Costa, C. I. Wassano, C. F. F. Angolini, K. Scherlach, C. Hertweck, and T. P. Fill, "Antifungal potential of secondary metabolites involved in the interaction between citrus pathogens," Scientific Reports, vol. 9, Art. no. 18647, 2019, doi: 10.1038/s41598-019-55204-9.

[2] A. M. Kanashiro, D. Y. Akiyama, K. C. Kupper, and T. P. Fill, "Penicillium italicum: An underexplored postharvest pathogen," Frontiers in Microbiology, vol. 11, Art. no. 606852, pp. 1–17, 2020, doi: 10.3389/fmicb.2020.606852.

[3] I. Kosalec, S. Pepeljnjak, and M. Jandrlić, "Influence of media and temperature on gliotoxin production in Aspergillus fumigatus strains," Archives of Industrial Hygiene and Toxicology, vol. 56, pp. 269–273, 2005.

[4] G. I. Mohammed, A. F. Al-Rubaye, and I. H. Hameed, "Using GC-MS technique for analysis of bioactive chemical compounds of Penicillium italicum and determination of its antimicrobial activity," Indian Journal of Public Health Research & Development, vol. 9, no. 3, pp. 552–557, 2018.

[5] G. Oliver, A. P. Holgado, and R. Salim, "Dimorphism in Candida albicans: Effect of cycloheximide and acridine orange on germ tube formation," Mycopathologia, vol. 79, pp. 43–47, 1982.

[6] J. Webster and R. Weber, Introduction to Fungi, 3rd ed. Cambridge, U.K.: Cambridge University Press, 2007.

[7] M. E. Zain, "Impact of mycotoxins on humans and animals," Journal of Saudi Chemical Society, vol. 15, pp. 129–144, 2011, doi: 10.1016/j.jscs.2010.06.006.