

## New Record of *Penicillium vanoranjei* in Iraq تسجيل جديد للفطر *Penicillium vanoranjei* في العراق

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### Abstract

The present study aimed to identification of *P. vanoranjei* based on microscopical and cultural method as a new record isolate in Iraq. *Penicillium vanoranjei* is a new species in Iraq that produce an orange production pigment after complete growth on the culture medium. Location, the specimen collected from women patient admitted to Al Jimhory Hospital in Samarra city and lived in Al Jilam township during December/ 2016 in Salah Al Deen Governorate- Samarra City. Morphological and microscopical characteristics appeared that the top view of the culture slightly raised in the center; mycelia was white near the margins, the reversed view appeared Dark brown. Microscopic examination illustrates that the Conidiophores were monoverticillate (unbranched) *Penicillium vanoranjei*.

الملخص

نوع جديد سجل لأول مرة في العراق للفطر *Penicillium vanoranjei* حصل عليه بعد التصبغ والتنمية في الوسط الزرعي من النساء المرضي المراجعات الى مستشفى سامراء العام في مدينة سامراء/ صلاح الدين، واللواتي يسكن منطقة الجلام الريفية و يعانين من اصابة فطرية خلال شهر كانون اول-2016. الصفات المظهرية والتشخيصية اظهرت ارتفاع النمو بشكل قليل عند المركز مع خيوط فطرية بيضاء بالقرب من حافة المستعمرة مع اللون البني الداكن عموما للمستعمرة. كما اظهر التشخيص المجهرى بان الكونيدات السبورية احادية غير متفرعة ومطابقة للفطر *Penicillium vanoranjei*.

كلمات مفتاحية: *Penicillium vanoranjei*, تسجيل جديد، *Penicillium* البرتقالي، اختبار زرعي

### Introduction

*Penicillium vanoranjei* is relatively rare species isolated for the first time in Tunisia the maine characterized by colonies dominated by bright orange sclerotia and production of orange colonies in culture. The history of the name (Dutch = oranje) related to Willem-Alexander Claus George Ferdinand, 'Zijne Koninklijke Hoogheid de Prins van Oranje' (translated from Dutch as: 'His Royal Highness the Prince of Orange') and his family, to coincide with his coronation also the name of this fungus when isolated from Tunisia soli was named as a tribute to the Dutch royal family, specifically His Royal Highness the Prince of Orange which consider the first isolation in the world. In May 2014, the International Institute for Species study listed *P. vanoranjei* as one of the "Top 10 News Species" named in 2013. "*Penicillium vanoranjei* C.M. Visagie, J. Houbraken & R.A. Samson, Persoonia 31: 46 (2013) [MB#803782] [1].

### Materials And Methods

#### Sample Collection

During the period of December/2016 total of 100 scraped samples were collected from patients (18 to 66 years old of both genera) suspected to suffer from dermatophytosis disease, (as clinically identified by a physician) from the Dermatology unit of Al Jimhory Hospital in Samarra city. The samples were subjected for direct examination using 10% KOH specimen were placed on a microscopic slide, with the few drops of 10% KOH, a cover slip added and warmed over a small flame just before boiling. The slide was examined under the low power and high dry objectives to detect the presence of the fungi and their hyphae[1]. Scraping of each samples culturing on the sabouraud dextrose agar SDA supplemented with 0.04 mg/ml chloramphenicol to inhibit the growth of bacteria, Potato Dextrose agar (PDA), Czapek-dox medium medium, and Malt extract Agar MEA then incubated at 30 °C and examined for 7-14 days [2,3,4,5,6]. A small piece of transparent-adhered tape was touched to the surface of the suspected colony, and then adhered to the surface of a microscope slide to

which a drop of lactophenol cotton blue was added. Shape and arrangement of the spores were examined microscopically [3].

### Key Classification

The following reference was used for classification and identification the *Penicillium vanoranjei*: *Penicillium* [4]: The hyphomycete genus is characterized by macronematous penicillate conidiophores, the ultimate branches of which are verticillate phialides while the penultimate branches are termed metulae

### Isolates Preservation

Pure cultures of isolates were maintained in SDA and PDA slant media and glycerol. These were placed in 4 °C as stock cultures. The sub culturing was done every three months [3].

### Results and Discussion

The results of the current study showed that on the cultured samples related to the *P. vanoranjei* and confirmed after microscopic examination identified as a new record in Iraq, *vanoranjei* isolated from clinical sample from women with 45 years old-suffering from ringworm *Tinea unguium* in her finger as show in Figure (1), lived in the farm (Al-jilam) around Samara city.

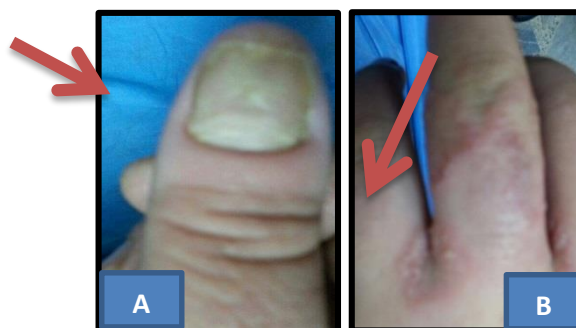
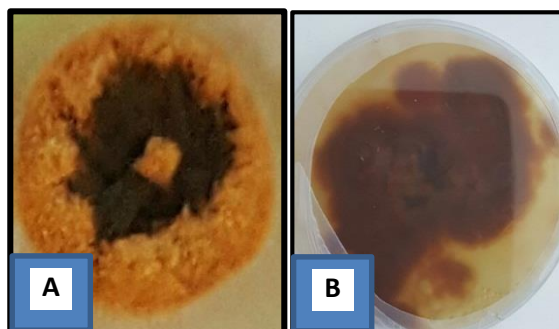


Fig. (1): A: *Tinea unguium* B: Ringworm in the hand which *Penicillium vanoranjei* isolated related to the same finger of the patients

*P. vanoranjei* first recorded in 2013 [5] and to the present time of current study no associated records, and the information of the first registration documented in different code numbers and published in [www.Mycobank.com](http://www.Mycobank.com) [6] C.M. Visagie, J. Houbraken & R.A. Samson Literature: Visagie C.M.; Houbraken J.; Rodrigues C.; Silva Pereira, C.; Dijksterhuis J.; Seifert K.A.; Jacobs K.; Samson R.A. 2013. Five new *Penicillium* species in section *Sclerotiora*: a tribute to the Dutch Royal Page: 46 Year of publication: 2013 Date public: 2013-04-12 Type specimen or ex type: CBS 134406 = DTO 99H6 = AHS3SF\_13 GenBank accession numbers to ITS barcodes and alternative identification markers are provided (BT =  $\beta$ -tubulin; CMD = calmodulin). Visagie, Houbraken & Samson (this study). (MB803782). — Herb.: CBS-H 21145. Ex-type: CBS 134406 = DTO 99H6 = AHS3SF\_13. ITS barcode: KC695696. (Alternative markers: BT = KC695686; CMD = KC695691).

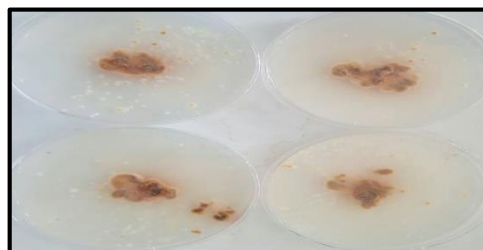
The results of cultural examination on SDA showed overview of *P. vanoranjei* description pattern surface of top view appeared slightly raised in the center; mycelia was white near the margins and the reversed view appeared Dark brown as illustrated in Figure (2).



**Fig. (2): Overview of colony characters of *Penicillium vanoranjei* grown on SDA at 30°C for 7days A: Top view and B: Reversed view.**

Colonies of *P. vanoranjei* on Potato Dextros Agar slightly raised at centre, radially sulcate, abundant bright orange sclerotia produced; margins

low, narrow, entire; mycelia white near margin, orange elsewhere; texture floccose; sporulation absent to sparse, conidial colour cannot be determined; exudate bright orange, reverse pigmentation brownish as presented in Figure (3).



**Fig. (3): Overview characters of *Penicillium vanoranjei* colony grown on PDA at 30°C for 7days.**

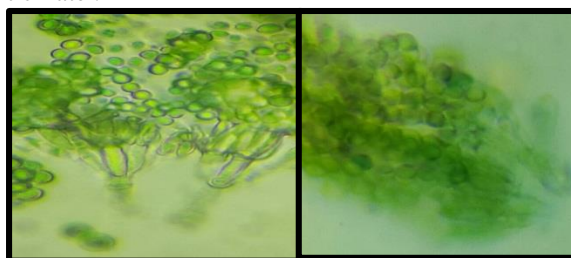
The growth progress of *P. vanoranjei* to 14 day lead to production of bright orange color as in Figure (4).



**Fig. (4): Production of bright orange color by *Penicillium vanoranjei* when grown on SDA at 30°C for 14days**

There are number of *Penicillium* species which have the ability to produce pigments in high yields. Amongst them, many species have attracted special attention because they have the capability of producing different coloured pigments showing high chemical stability [5,7,9]. May be used for industrial and medical applications but the bright orange color of *Penicillium vanoranjei* now under study to produce the pigment in optimum condition and subjected it for different application.

Microscopic examination of *Penicillium vanoranjei* shown in Figure (5) Illustrated, that the Conidiophores are unbranched monoverticillate .



**Fig. (4): Microscopic feature of *Penicillium vanoranjei* stained with Lactophenol cotton blue (40X), monoverticillate conidophores.**

The taxonomy of the fungus *Penicillium vanoranjei* confirmed by profesour Dr Abdulkarem Jasim Hashim/ Baghdad university /College of Science/ Biotechnology Department: Kingdom: Fungi, Phylum: Ascomycota, Pezizomycotina, Class: Eurotiomycetes, Eurotiomycetidae, Order:Eurotiales, Family:Trichocomaceae, Genus:Penicillium, species *Penicillium vanoranjei* [4,10,11].

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#### References

1. Emmons, C. W., Binford, C. H., Utz, J. P. and Kownchung, K. J. (1977). Medical Mycology. 3<sup>rd</sup> Ed. Lea and Febiger, Philadelphia.
2. Jawetz, E.; Melnick, J. and Adelberg, E. A. (1991). Medical Microbiology. 20<sup>th</sup> Ed., Middle East edition. P: 532-546.
3. Midgley, G., Clayton, Y. M. and Hay, P. R. J. (1997). Diagnosis in color medical mycology. 1<sup>st</sup> Ed., Mosby-Wolf.
4. Nouri M. A., Al-Halbosi, M. M.F., Dheeb, B. I., Hashim, A.J. Cytotoxicity and genotoxicity of gliotoxin on human lymphocytes in vitro. Journal of King Saud University – Science (2015) 27, 193–197
5. V Domsch, K.H., Gams, W. and Anderson, T. (2007) .Compendium of soil fungi London: Academic Press.
6. Isagie, C.M., Houbraken, J., Rodriques, C., Silva Pereira, C., Dijksterhuis, J., Seifert, K.A., Jacobs, K., and Samson, R.A. (2013). Five new *Penicillium* species in section *Sclerotiora*: a tribute to the Dutch Royal family. *Persoonia*. 31:42-62.
7. Dheeb, B.I. (2013). Immunohistochemical study of Tumor Necrosis Factor- $\alpha$ (TNF-  $\alpha$ ) expression in lung, liver, and spleen during aspergillosis infection. *BMC genomics* 15 (2), P71
8. <http://www.mycobank.org/BIOLOMICS.ASPX?TABLEKEY=14682616000000067&REC=511357&FIELDS=ALL>
9. Dheeb, B.I., Mohammad, F.I., Hadi, Y.A., Abdulhameed, B.A. (2013). Cytotoxic effect of aflatoxin B1, gliotoxin, fumonisin B1, and zearalenone mycotoxins on HepG2 cell line in vitro. *Int. J. Adv. Res.* 1 (8), 355–363.
10. Hajjaj, H., Blanc, P., Groussac, E., Uribelarrea, J.L., Goma, G., Loubiere, P. (2000). Kinetic analysis of red pigment and citrinin by *Monascus ruber* as a function of organic acid accumulation. *Enzyme Microb. Technol.* 27: 619-625.
11. Dheeb, B.I. (2015). Antifungal Activity of Alkaloids and Phenols Compounds extracted from black pepper *Piper nigrum* against some pathogenic fungi *Jornal of Biotechnology Research Center* (2015) 9, 46-54.