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Isolation And Identification Of The Fungus Monascus Purpureus From Imported Currants As a New Record In Iraq

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Isolation and identification of the fungus Monascus purpureus from imported currants As a new record in Iraq

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ABSTRACT

This study was conducted in the Laboratory of Fungus in the Department of Biology / College of Science / University of Qadisiyah to isolate and diagnose some insulation from fungi isolated from imported dried fruits (currants) in Qadisiyah province, Iraq. The isolations were diagnosed both morphologically and microscopically using the classification keys and to confirm the appearance and microscopic diagnosis diagnosed using polymerase chain reaction(PCR), And determine the sequence of nitrogen bases (Nucleotide sequence(of compound DNA products using ITS1 and ITS4. The results of the nucleotide sequence analysis of DNA (PCR product) compounding innate isolation and using BLAST to compare with data available at the U.S. National Center for Biotechnology Information (NCBI) have shown that this isolation belongs to the type Monascus purpureus. By comparing the sequence of nitrogen bases of isolated M. purpureus fungus in this study, it was found that there was a 100% similarity to many of the M. purpureus fungus isolates previously registered at the National Center for Biotechnology Information (NCBI), including those diagnosed in China (MT361825, MK359689, MW581230) and Japan (AB477248).

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1. Introduction

Fungi possess a high potential to produce secondary metabolites (SMs) of the polyketide family [3], some of them are well characterized because of their biological activities or toxicity [6]. The genus *Monascus* belongs to the family Aspergillus and orders Eurotiales. It contains 36 species; however, many of them are considered synonymous .The genus *Monascus* belongs to the phylum Eumycota, subphylum Ascomycotina, class Plectomycetes, order Eurotiales, family Monascaceae and currently comprises nine species, i.e. *M. floridanus*, *M. pallens, M. pilosus, M. purpureus, M. ruber, M. sanguineus, M. eremophilus, M. luis porras and M. argentinensis*, For which there are a number of synonyms[10]. At present, one of the most popular natural color- producing fungus strains for food application is *M. purpureus* because of its remarkable ability to produce many different types of pigments and secondary metabolites of polyketides structure. It has recently become a popular dietary supplement because of the discovery that it contains many bioactive constituents which have pharmaceutical benefits.These constituents include monacolins, pigments, dihydro monacolins, citrinin, γ- Aminobutyric acid and dimerumic acid [4] . However, under certain conditions some strains of *Monascus* produce a secondary metabolite citrinin which is toxic and which can cause a safety problem for consumption [2]. These fungi are a source of various polyketide-type secondary metabolites and are famous for their fermented products. *M. purpureus* contains unsaturated fatty acids, sterols, monacolin and azaphilone pigments. It has been reported that these compounds are effective in lowering cholesterol, as well as in the treatment of diabetes, cardiovascular diseases, and some cancers [5]. In this study, one of the species belonging to the genus *Monascus, M.purpureus*, was isolated from dried fruits (currants) and this species was diagnosed morphologically and microscopically and these two diagnoses were confirmed through the molecular diagnosis procedure using polymerase chain reaction technology(PCR) where the results of this analysis proved that this isolation belongs to type *M. purpureus*. By comparing the sequence of nitrogen bases from isolated *M. Purpureus* fungus in this study, it was found that there was a 100% similarity to many *M. purpureus* fungus isolates previously registered at the National Center for Biotechnology Information (NCBI). There is no evidence that this species has ever been diagnosed in Iraq, so this species was first recorded in Iraq in this study and no one has ever recorded it.

2.METHODS

Samples of imported dried fruits (currants) were collected from the markets and shops of Diwaniyah provincial center and districts for the period (August-December 2020). The contaminated fungi of dried fruits used in this study were isolated where each type of sample was divided into two groups, the first group included samples that were superficially sterilized by sodium hypochlorite at a concentration of 1% and for 3 minutes after which they were washed with distilled water three times to remove the effect of sterilization .The second group was washed with only distilled water, Parts of sterile and unsterilized samples were grown in petri dishes container on the PDA food medium by three repeaters per sample and the dishes were incubated for 7 days at 25˚C temperature, during which time the growth of fungi was followed up and the dishes were examined to find out the developing fungi, after which the appearance and microscopy of fungal isolations was diagnosed and confirmed this diagnosis molecularly. It is worth mentioning that the diagnosis of mushrooms *M. purpureus* was done with the help of references and scientists in this field, and was confirmed through the information available at the National Center for Biotechnology Information (NCBI) and by the Iraqi workman Dr. Samir Khalaf Abdullah that this type was recorded for the first time in Iraq in this study.

3.Results and discussion

Phenotypic and microscopic diagnosis

Fungi isolated to the level of genus and species were diagnosed based on the external appearance of the colony Morphological features such as color, shape and colony texture, and some microscopic features such as the size, shape hyphae and conidia, according to the classification bases adopted and using the classification keys contained in[11,1] . *M. Purpureus* colony shape is velvety and its color varies according to its age as the color of the colony after 5 days of cultivation ranges from creamy to orange while when it is 7 days old it is red and when it reaches the age of more than 7 days it appears in dark red and shown in Figure (1).

Figure 1:(A) The color and shape of *M. purpureus* isolated from currants.

(B) The color and shape of *M. purpureus* after seven days.

(c) The color and shape of *M. purpureus* after more than seven days.

M. purpureus is distinguished by its usually spherical ascospores[9], which are 5 microns in diameter or ovoid (6×5 microns). During the early stages, the young part of the *M. purpureus* mycelium is white. However, this rapidly changes to a rich orange and later to a distinctly rich red color, reflecting the increasing acidity of the medium and the production of red-orange hyphae. A deep crimson color is found at the substratum as the culture ages[7].

Under the microscope, the species of Monascus are normally characterised by nonostiolate ascomata arising singly at the tip of stalk-like hyphae scattered on the mycelium, and an ascomatal wall composed of two distinct layers, an inner layer which results from the swelling of the tips of the stalk-like hyphae forming a vesicle- like structure and an outer layer consisting of hyphal branches growing out from the base and fusing with the inner vesicle As shown in Figure (2).The morphological characteristics of the colonies (including the size, color, shape, and aerial hyphae) and microscopic characteristics(including hyphae, conidia, cleistothecia, and ascospore) were observed using the protocol described[8]. It has a velvety shape as observed under the microscope Hyphae and mycillum which is septate as conidia and which represents asexual phase, conidia are aleuroconidia that develop in conidiophores and occur either single as cells or in chains. As shown in figures (2,3).

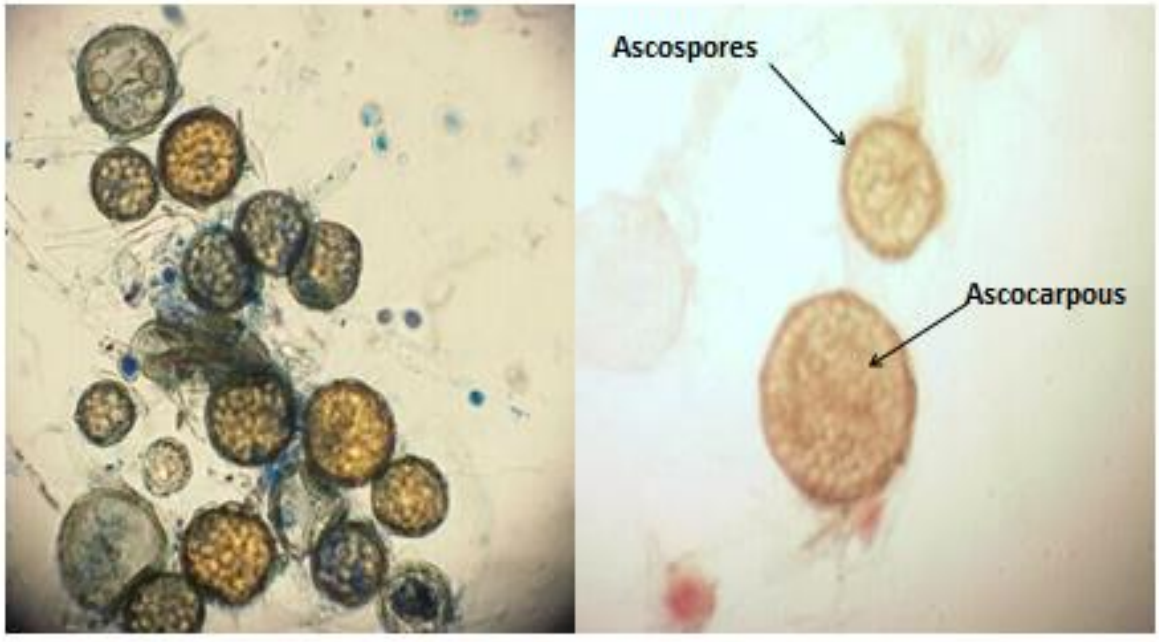

Figure2 Left:: arrangement of conidiophores; Right:Installation of Ascospores and Ascocarpous for *M. Purpureus.*

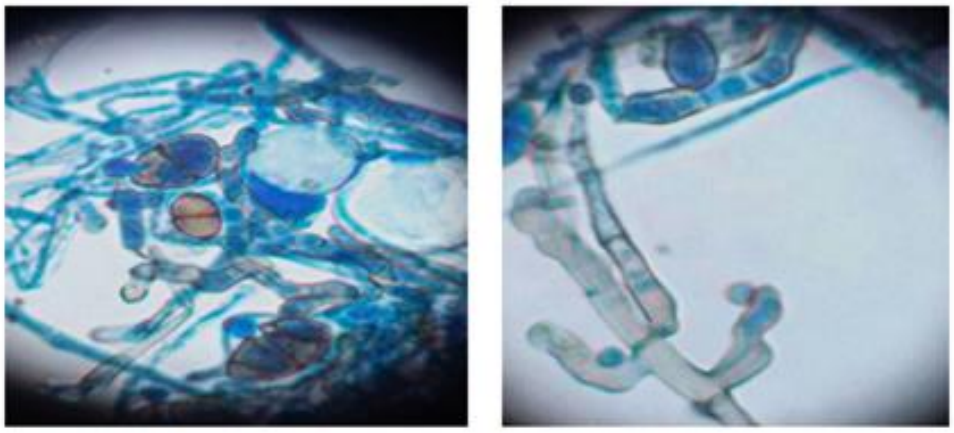

Figure3: Left: mycelium of *M. Purpureus*; Right: Hypha septate.

4.Molecular diagnosis of isolated fungus *Monascus purpureus* using polymerization chain reaction technology (PCR).

The results showed that PCR-amplified products could be multiplied by 550 pairs of nitrogen bases (Base pairs, bp) by PCR) and with front(ITS1) and reverse (ITS4) -type Primer pairs (Figure 4**).**

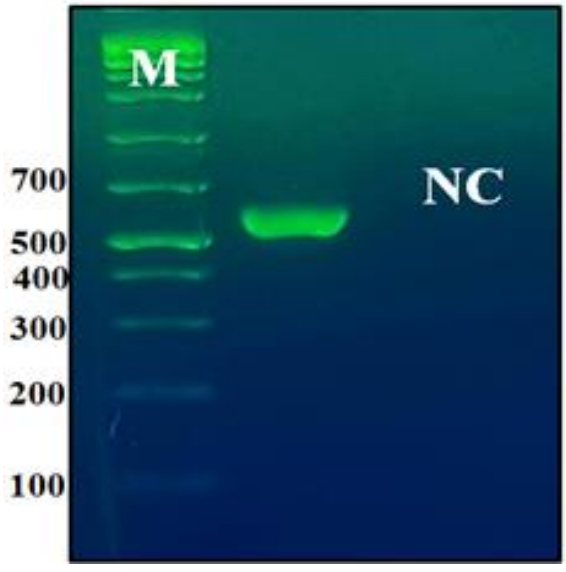

Figure 4: PCR product multiplier using PCR form of isolated fungus isolation in this study from raisins and using its ITS 1 and ITS4 pairs of Primer. M=(Molecular-weight size marker), NC: Negative comparison.

The results of the nucleotide sequence analysis of DNA (PCR product) compounding innate isolation and using BLAST to compare with data available at the U.S. National Center for Biotechnology Information (NCBI) have shown that this isolation belongs to the type *M. purpureus*. By comparing the sequence of nitrogen bases from isolated *M. purpureus* fungi in this study, it was found that there was a %100 similarity to many of the *M. purpureus* fungi isolates previously registered at the National Center for Biotechnology Information (NCBI), including those diagnosed in China (MT361825, MK359689, MW581230) and Japan (AB477248) (figure 4 and figure 5).

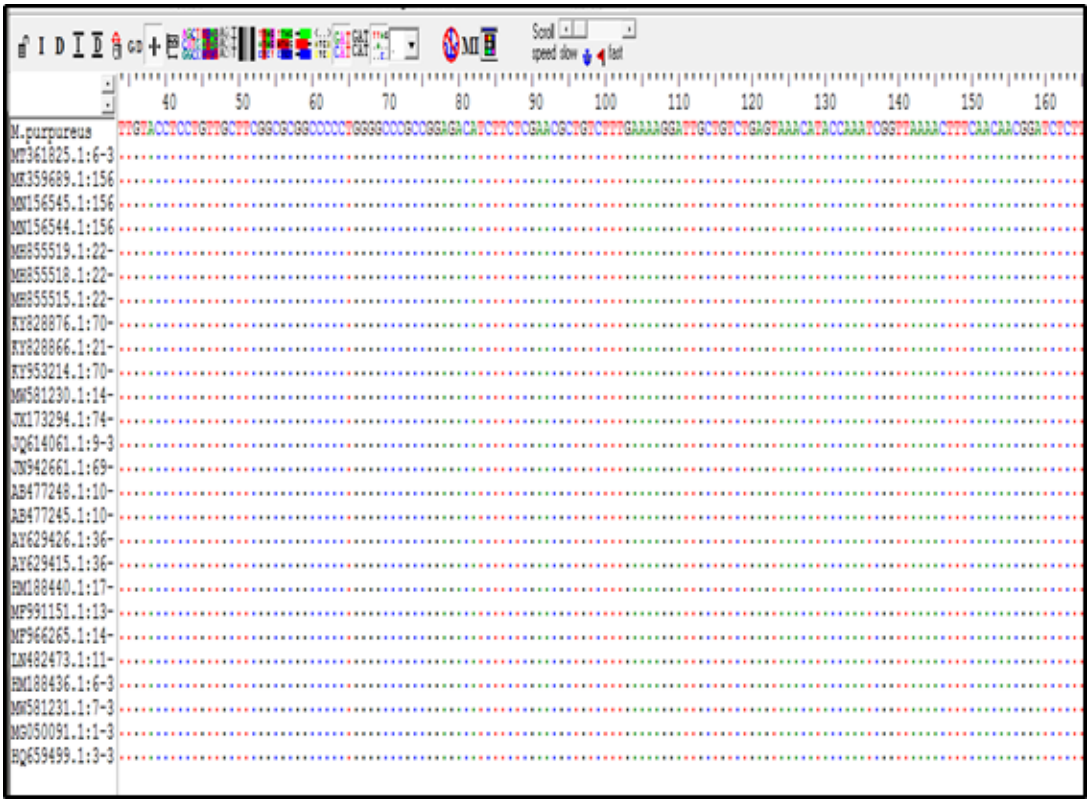

Figure5: Similarity in sequence alignments of DNA products (PCR-amplified products) multiplied by the isolation of isolated *M. purpureus* fungi in this study and other isolations of the same mushrooms previously registered at the National Center for Biotechnology Information (NCBI).

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