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Phenotyping and Genotyping of *Saprolegnia parasitica* isolated from Diwaniyah River-Iraq

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Abstract

The current study included a study of the spread of *Saprolegnia parasitica* in the water of the Diwaniyah River, water samples were collected for three months from the Al-Furat Bridge area for three months August, September, and October, isolation was done by bait method. After that, diagnosis was made by the traditional method adopted to explain the appearance and was supported by molecular diagnosis by PCR. The results showed that the fungus *S.parasitica* was present in varying numbers and percentages according to the months of the study, the numbers increased with decreasing temperatures, as they were month of October was the highest month in terms of the number of isolates, as it reached 10, representing a percentage of 46%, while the month of September had 8 isolates, a percentage of 36%, and the month of August. It was the lowest, as it was 4, with a rate of 18%. The molecular study appeared that the diagnosis of all isolates was identical to the traditional diagnosis, which relies on the appearance of the fungus in plates and under an optical microscope, where the bands were at 568 bp.

Keywords:- Saprolegnia, parasitica , Diwaniyah ,river and molecular .

1. Introduction

The genus Saprolegnia is one of the most widespread fungal genera. It is an aquatic fungus that lives in rivers, lakes, and even seas. This genus belongs to the fungi of the Phylum Oomycota, whose spores have two flagella, one of which is feathery and the other is simple.[1] The Saprolegniaceae family is one of the well-known fungal families and is called water mold. It grows in water and live by analyzing organic materials, which helps in analyzing elements in nature, and some of them parasitize on algae, arthropods, fish, and their eggs, they are called fish mold[2].

Saprolegnia parasitica is a fungus spread in fresh water that attacks fish and their eggs. It possesses virulence factors that enable it to colonize the body of the host. It possesses keratinase, which decomposes fish scales and fins, which are a food substance for this fungus. In Iraq many studies carried out to documents the spread of this fungi in rivers and marshes[3-5].

Diwaniyah river is an extension to Euphrates river. It enters Diwaniyah Governorate at the city of Al-Suniya and ends at the city of Al-Hamza[6]. It is the primary source of drinking and irrigation water in the governorate and contains a wide biological diversity of plants, animals, fish, as well as various microorganisms.

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Many fungi live in this river, some of which come to it accidentally, especially saprophytic fungi, which work to break down organic materials into their primary elements, which helps in recycling the elements in nature, while there are indigenous aquatic fungi that spend their entire life cycle in water, such as *Saprolegnia* spp, and many have been mentioned researches[7, 8]. It is an important biological indicator that gives an idea about the level of water pollution. This fungus is characterized by an undivided mycelium and a wide, transparent hypha. It reproduces asexually by division, and zoospores are produced inside elongated sporangia, which gives a two-flagellated, pear-shaped spore that later turns into a kidney-shaped one. The sexual reproduction by gametangial contact and the sexual spore is Oospore[9].

S. parasitica is one of the primary pathogens for adult and small fish, as well as for fish eggs. The infection may appear on fish in the form of white or gray spots. The fungus is characterized by its rapid growth and wide branching hyphae. The fungus can be isolated on food media using baits such as cooked millet and flax seeds, or by insect body[10]. It grows on solid media in the laboratory in the form of branched white cotton mycelium. The current study came with the aim of shedding light on the fungus *S. parasitica* and the extent of its spread in the Diwaniyah River and profiling it phenotypically and genetically. The study includes collecting water samples and isolating the fungus using grafts, as well as studying the fungus phenotypically and genetically.

2. **Material and methods :-**

Samples collections

Samples were collected by opaque bottle from the water of the Diwaniyah River for the period from October to November of 2023, where one liter of water was brought from a depth of 10, 20 and 30 cm, from the banks of the river and from the middle, then placed in a cooled box and transported as quickly as possible to the laboratory to conduct experiments. On it, where the collection was semi-weekly and in the morning near the Al-Furat neighborhood bridge[11].

Isolation and Identification of fungi

Isolation was done by bait method, where millet seeds were used, which were cooked well until ripe and distributed in petri dishes containing river water sterilized by an autoclave. After cooling, the river water was shaken well, then 1 ml was added to each dish containing the millet seeds and incubated at a temperature of 28°C until emergence. White growths around the seeds[12]. Phenotypic analysis was carried out based on the shape of the fungus growing on the seeds, as well as the microscopic characteristics of the hyphae, sporangia, spores and other incorporeal characters, and by comparison with taxonomic keys and atlases[13-15].

Genetic study

DNA extracted After freshly harvested fungal mycelium were transferred to a 1.5 mL centrifuge tube, the fungal strains were sub-cultured onto sterile Sabouraud dextrose agar plates and incubated at 22ºC for three to five days. Following this, DNA was extracted from each sample using a Wizard Genomic DNA purification kit (Promega, USA) with some modifications. The yield of DNA was then stored at -20° C until it was needed. DNA Purity and Concentration Following the manufacturer's instructions, the nanodrop instrument was used to quantify the concentration and purity of the purified DNA (Act Gene NAS99) In summary, 3µl was aspirated using special tips and placed into the machine's designated socket. The refractive index of the DNA was measured using wave lengths of 260 and 280 nm. concentration of DNA Polymerase Chain Reaction PCR The primers (ITS4) to detection

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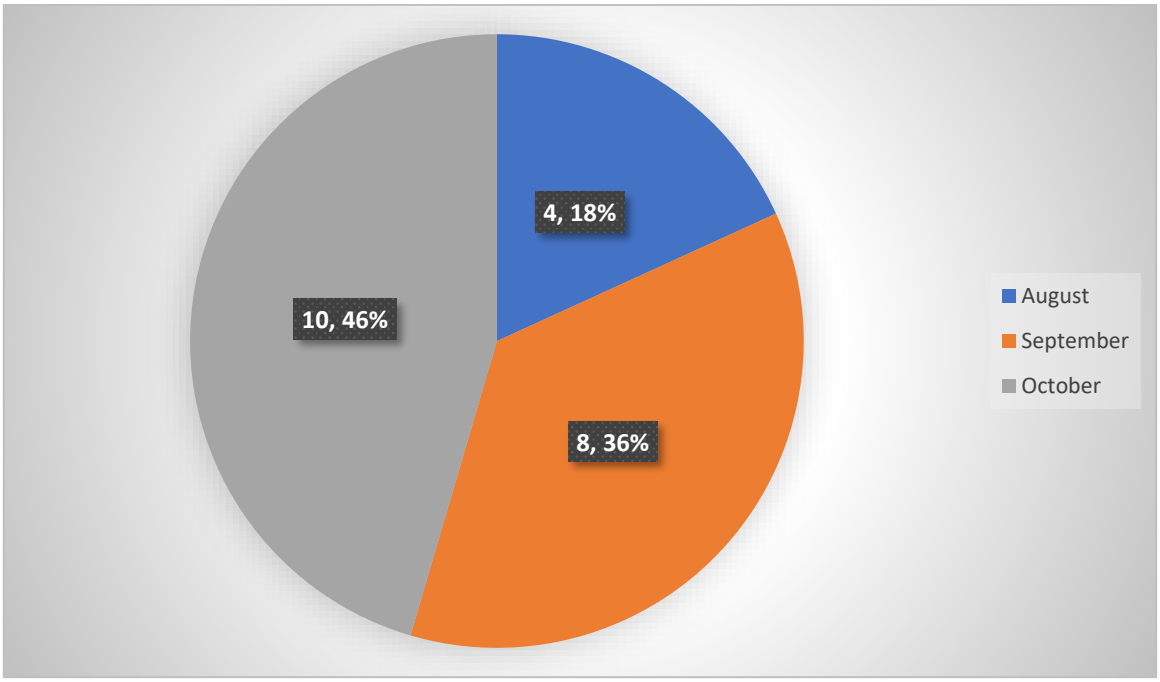
Saprolegnia parasitica were diluted by adding nuclease free water according to the manufacturer instructions and .the primers used in study was ITS4(TCCTCCGCTTATTGATATGC). [16]

Results and discussion

The results reached Figure 1 that there is a difference in the numbers and percentages of isolates according to the months of collection from the Diwaniyah River, where the month of October was the highest month in terms of the number of isolates, as it reached 10, representing a percentage of 46%, while the month of September had 8 isolates, a percentage of 36%, and the month of August. It was the lowest, as it was 4, with a rate of 18%. The reason for this discrepancy is that the fungus thrives, grows, and reproduces at relatively low temperatures of about 20-25 degrees Celsius. This is consistent with what previous studies mentioned, that the fungus increases and grows in the cold months in Iraq and decreases in the summer[2, 11, 17].



figure(1) numbers and percentage of *Saprolegnia parasitica* isolated from Diwaniyah river

Ten isolates of the fungus *Saprolegnia parasitica* were subject to PCR and the PCR products were electrophoresed for the purpose of molecular diagnosis, and the diagnosis of all isolates was identical to the traditional diagnosis, which relies on the appearance of the fungus in plates and under an optical microscope, where the bands were at 568 bp. The use of the ITS region is one of the matters widely accepted in the field of diagnosis, as it is considered highly stable and gives accurate results, especially when using high-quality primers from original source[16, 18, 19]. The spacer DNA known as internal transcribed spacer (ITS) is located between the large-subunit and small-subunit ribosomal RNA (rRNA) genes on a chromosome, or it may be the homologous transcribed region in the polycistronic rRNA precursor transcript. There is just one ITS in bacteria and archaea, and it is situated in between the 16S and 23S rRNA genes. In eukaryotes, on the other hand, there are two ITSs: ITS1 is situated between 18S and 5.8S rRNA genes, and ITS2 is situated between 5.8S and 28S (or 25S in plants) rRNA genes.

ITS1 and ITS in bacteria and archaea are equivalent. While ITS4 genes are useful and crucial for fungi, they have been used to diagnose many fungi such as Candida, Aspergillus, and others.[20-22]

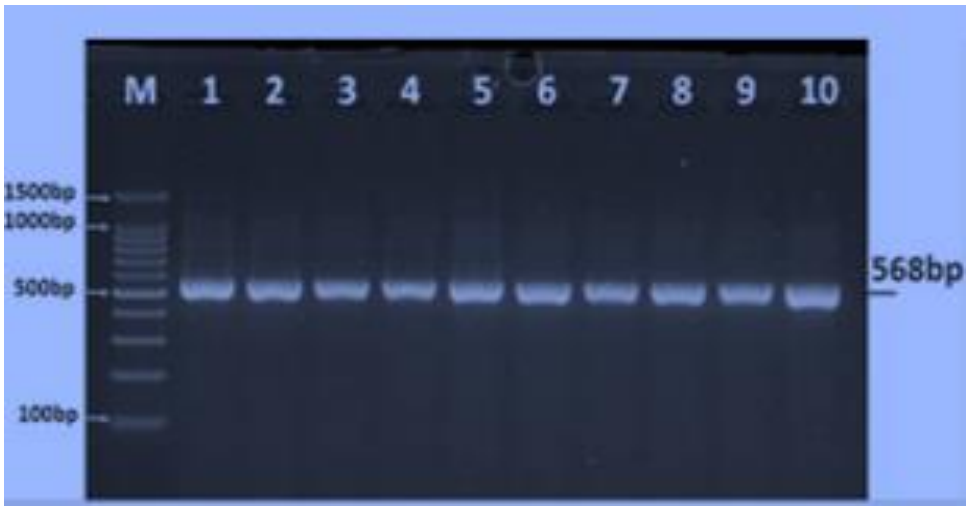


Figure (2) gel electrophoresis of PCR product 18S rRNA gene ITS4 region for identification of *S.parasitica* ,then numbers is the isolates and M is the ladder (1500-100pb)

Conclusion:- The fungus *S. parasitica* is present in the Diwaniyah River, and its numbers increase with decreasing temperatures, as its highest percentage was in October. The traditional method of diagnosis was identical to the diagnosis using the PCR method, based on ITS genes.

Recommendation :- Conducting extensive studies on biological diversity in the Diwaniyah River with the aim of knowing the relationship between fungi and other organisms.Studying the spread of mushrooms throughout the year and the relationship between them and climatic and environmental changes.Studying the effect of fungi on fish and their eggs in the river.

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