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Isolation and Diagnosis of Some Types of Fungi Using PCR Technology for Infection by (*Musca locala* L.) From some Areas of Anbar Governorate

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Abstract:

This study revealed the presence of several fungal species, some of which are saprophytic, and others are pathogenic, in addition to the presence of fungi used in biocontrol in soil samples collected from six different regions of Anbar province. The identified fungal genera include *Metarhizium anisopliae*, *Trichoderma harzianum*, *Penicillium*, *Fusarium spp.*, *Rhodotorula*, *Rhizopus*, *Candida spp.*, and *Aspergillus flavus*. Confirmation of the diagnosis of the pathogen of the housefly (*Musca domestica*) using PCR technique indicated the presence of different genera of fungi at various concentrations, with the highest frequency observed for *Aspergillus flavus* (78.2%) and the lowest frequency for *Trichoderma harzianum* (6.5%). Among the isolated fungi, pathogenic fungi belonging to the genus *Metarhizium anisopliae* and *Trichoderma harzianum* were identified. Molecular diagnosis of the two pathogenic fungi was conducted using the polymerase chain reaction (PCR) technique, and the purity of the isolates ranged between 1.69 and 1.94, with concentrations ranging from 34.03 to 52.10 ng/ml. The newly isolated strains were deposited in the NCBI Gene Bank under the researchers' names.

Keywords: Fungi, Insects, *Trichoderma harzianum*, *Metarhizium anisopliae*, PCR

Introduction

The kingdom of fungi is one of the most diverse and ancient branches of the tree of life, comprising approximately 2-5 million species that play vital roles in terrestrial and aquatic ecosystems. It is believed that fungi co-evolved with plants, and strain identification aids in understanding the evolution, pathways, characteristics, and biology of fungi in general (1)

Fungi and insects, as two of the most ubiquitous organisms in nature, have various impacts on overall environmental and human activities, resulting from their mutual interactions. They establish a wide range of mutualistic, symbiotic, and antagonistic relationships. Pathogenic fungi are natural enemies of insects, with some strains of certain species being important natural enemies of widespread insect pests in natural and agricultural ecosystems, thus playing a significant role in biological control and maintaining the ecological balance in these systems (2).

Soil is a suitable habitat for fungi and other microorganisms, protecting them from various environmental factors, especially UV radiation, and

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mitigating the negative effects of other factors. The type and properties of soil, such as its chemical and physical characteristics, influence the diversity of genera and dominant species within it (3). The ability of any fungus to survive in soil and adapt to surrounding environmental conditions depends on the availability of organic matter, soil acidity (pH), moisture content, aeration, and type of cultivation (4).

Pathogenic fungi encompass numerous species belonging to multiple genera (5). *Metarhizium anisopliae* and *Trichoderma harzianum* are among the most prevalent species of entomopathogenic fungi in soil (6), affecting significant groups of insect pests (7). They are present year-round in soil, decomposing plants, seeds, and grains, but only a few known species are significant pathogens causing opportunistic diseases in humans (8). *M. anisopliae* is a known entomopathogenic fungus active against many insect pests and an effective biocontrol agent(9) However, *M. anisopliae* is one of the most widespread fungi and is one of the most important deficient fungi that infect insects and cause various diseases such as green muscardine disease, which is so named because its spores that will cover the bodies of dead insects are green in color and this pathogenic fungus is often found in the soil and infects all soil insects, but it can also infect insects that are not natural hosts for it, such as mosquitoes, and is one of the most prominent fungi that successfully control insect pests (10). Trichoderma is a filamentous fungus that has been widely studied and used as a biological control agent for pathogenic fungi due to its ability to parasitize fungi. In recent years, studies have focused on investigating the possibility of using it as a biological control agent for insect pests, whether directly or indirectly through parasitizing directly on insect pests and producing insecticidal secondary metabolites, anti-nutritional compounds, and repellent metabolites, and through attracting natural enemies or parasitizing insect symbiotic microorganisms. By producing secondary metabolites of insecticides, anti-nutritional compounds, and repellent metabolites, and by attracting natural enemies or indirectly parasitizing insect symbiotic microorganisms, the use of Trichoderma against insect pests is seen as a future alternative in the development of sustainable agriculture. (11)

The housefly, *Musca domestica* L., is considered one of the most dangerous insects to human health (12), serving as a primary vector for many pathogenic microorganisms, such as typhoid, cholera, and anthrax (13). Due to the environmental and health problems associated with chemical pesticides, there is a need to search for environmentally safe alternatives for biological control that are non-pathogenic to humans (14).

DNA sequencing or molecular diagnosis (Polymerase Chain Reaction - PCR) is one of the best diagnostic techniques, enabling accurate, rapid, and specific discrimination between fungal species and subspecies, unlike traditional chemical and biological diagnostic methods. It is particularly useful for diagnosing fungi when fungal colonies lack diagnostic characteristics, are in early growth stages, or are even dead (15). PCR amplifies large quantities of targeted DNA fragments from one or a few mold spores through enzymatic repetition of a specific type of organism, allowing for DNA sequencing. It is now widely used in various fields of life sciences and medicine for its ease and effectiveness in identifying DNA-based species (14). DNA sequencing plays a significant role in identifying phylogenetic relationships among fungal species, making it an ideal scale for diagnosing fungal genera and species (16). Therefore, the current trend in diagnosis combines genetic and morphological approaches (17).

Given the scarcity of research on the molecular enumeration and diagnosis of entomopathogenic fungi in Anbar province and the potential of these fungi as a safe natural resource for the biological control of various insect pests (18), this research was conducted to confirm the presence of entomopathogenic fungi and their distribution in some agricultural and non-agricultural soils in Anbar province, as well as to determine their ability to

infect families, to assess their potential future application in practical biological control applications.

Materials and Methods

Sample Collection:

A total of 24 soil samples were collected, with 4 samples from each of the six different areas in the city of Ramadi, namely Agriculture, Abu Etha, 5 Kilos, Warar, Sufiya, and Hay Al-Dabat, ensuring sampling from both agricultural and non-agricultural areas (Table 1). Soil samples were excavated to a depth of 10-15 cm using a shovel after removing 1 cm of soil surface. Soil samples were collected at a rate of 100 grams of soil per sample. Samples were sieved using an alcohol-sterilized sieve to remove gravel and debris, then placed in polyethylene bags, with each bag labeled with the sampling location and date and transported to the laboratory for isolation and diagnosis following the method described by Liu *et al*. (19).

Isolation of Fungi from Soil:

Dilution Method:

1- Several DNA extractions were performed using the Wizard® Genomic DNA Purification Kit following the protocol provided by the American Promega Company, with the assistance of lyticase enzyme for fungal cell wall disruption. The extracted DNA was then subjected to electrophoresis according to Sambrook *et al*. (20).

2- Fungal colonies were cultured for isolation on Potato Dextrose Agar (PDA) medium for 5 days at 28°C. Fungal hyphae were harvested using an L-shaped needle and collected in 1.5 ml Eppendorf tubes, with each isolate collected individually and three replicates per isolate.

Decimal Dilution Method:

The decimal dilution method was used to obtain samples from fungal colonies for microscopic diagnosis according to the taxonomic keys provided by Domsch (21). The frequency (percentage) of fungal occurrence was calculated using the following equation: Fungal frequency percentage = (Number of fungal appearances / Total number of samples) × 100.

DNA Extraction:

Experiments for molecular analysis of fungal isolates were conducted at the Biotechnology Laboratory for Graduate Studies/Faculty of Agriculture/Anbar University.

Primers Used in PCR:

The forward primer ITS1 (5′-TCCGTAGGTGAACCTGCGG -3′) and the reverse primer ITS4 (3′ TCCTCCGCTT ATTGATATGC -5′) (TM=62.3) were used according to Chen *et al.* (22).

Sequencing Measurement:

PCR products were sent for Sanger sequencing using the ABI 3730XL DNA sequencer to Macrogen Corporation, South Korea. The obtained results were analyzed using certified analysis software.

Statistical Analysis:

Statistical Analysis System (SAS) software (version 2018) was used for data analysis to study the effect of different factors represented by concentrations and isolated fungi on the studied traits, such as fungal frequency percentage in different areas. Significant differences between the percentage frequencies were compared using the Chi-Square test at probability levels of 0.05 and 0.001.

Results and Discussion:

Isolation and Diagnosis:

Isolation of entomopathogenic fungi from soil samples revealed the presence of several fungi, including some entomopathogenic species as well as those used in biocontrol. These fungi belong to the following genera: *Metarhizium anisopliae*, *Trichoderma harzianum*, *Penicillium*, *Fusarium spp*., Rhodotorula, Rhizopus, *Candida spp*., and *Aspergillus flavus* (Tables 1-6). *Aspergillus flavus* exhibited the highest occurrence rate at 78.2%, particularly in the 5-kilo area. This aligns with previous studies such as Al-Salman *et al*. (23), indicating its significance as a major pathogen present widely in various soils worldwide, particularly in hot and dry regions of Asia and Africa, causing significant economic losses (24).

The presence of fungi belonging to the genera *Aspergillus*, *Fusarium*, and Penicillium suggests

their saprophytic nature, as they are not known for their entomopathogenicity (25). The highest occurrence of *Aspergillus flavus* was recorded in the Agriculture area at a frequency of 56% at a dilution of 10^{-3} (Table 1), while the lowest frequency of T. harzianum was recorded at 6.6% at a dilution of 10^{-4}. Similarly, the highest occurrence of *Aspergillus flavus* in the Bu'athah area was 57.1% at a dilution of 10^{-6} (Table 2), while the lowest frequency was 12.8% at a dilution of 10^{-4}. In the 5 Kilo area, the highest occurrence of *Aspergillus flavus* was 78.2% at a dilution of 10^{-5} (Table 3), while the lowest frequency of *Fusarium spp*. was 16.6% at a dilution of 10^{-6}. In the Warar area, the highest occurrence of *Aspergillus flavus* was 57.1% at a dilution of 10^{-5} (Table 4), while the lowest frequency of *Fusarium spp*. was 42.8% at a dilution of 10^{-5}. In the Sufiyah area, the highest occurrence of *Penicillium* was 37.5% at a dilution of 10^{-6} (Table 5), while the lowest frequency of Rhizopus was 6.5% at a dilution of 10^{-4}. Finally, in the Officers' area, the highest occurrence of *Aspergillus flavus* was 75% at a dilution of 10^{-5} (Table 6), while the lowest frequency of both Rhizopus and *Fusarium spp*. was 8.2% at a dilution of 10^{-6}.

Statistically significant differences ($P \leq 0.01$) were observed between isolated fungal species in terms of frequency percentage at a dilution of 10-3, with the highest frequency being 56% for Aspergillus flavus and the lowest being 12.8% for Candida spp. Likewise, at a dilution of 10^{-4}, significant differences ($P \leq 0.01$) were observed between isolated fungal species in terms of frequency percentage, with the highest being 53.3% for Aspergillus flavus and the lowest being 6.6% for *Trichoderma harzianum*. Significant differences ($P \leq 0.05$) were also observed between isolated fungal species at a dilution of 10-5, with the highest being 48.3% for Rhodotorula and the lowest being 16.1% for both *Penicillium* and *Metarhizium anisopliae*. However, no significant differences were observed at a dilution of 10-6. Overall, when comparing all dilutions and isolates, the differences were highly significant ($P \leq 0.001$), consistent with previous studies (26).

Table 1: Dilution, Isolated Fungi, and Percentage Frequency in the Agriculture Area

Dilutions	Isolated fungi	Number of fungi	Frequency percentage
10^{-3}	*Aspergillus flavus*	22	56
	Rhodotorula	6	15.3
	Penicillium	6	15.3
	Candida spp.	5	12.8
	Chi-Square-χ^2	--	20.598**
10^{-4}	*Aspergillus flavus*	16	53.3
	Candida spp.	7	23.3
	Rhodotorula	5	16.6
	Trichoderma harzianum	2	6.6
	Chi-Square-χ^2	--	12.793**
10^{-5}	*Rhodotorula*	15	48.3
	Trichoderma harzianum	11	35.5
	Penicillium	5	16.1
	Metarhizium anisopliae	5	16.1
	Chi-Square-χ^2	--	8.00*
10^{-6}	*Aspergillus flavus*	12	33
	Rhodotorula	10	27.7
	Metarhizium anisopliae	9	25
	Trichoderma harzianum	5	18.8
	Chi-Square-χ^2	--	2.889 NS
	Total: Chi-Square-χ^2 (P-value)	--	18.926 (0.0001)
(P≤0.001) ** ،(P≤0.05) *			

Table 2: Dilution, Isolated Fungi, and Percentage Frequency in the Bu A'tha Area

Dilutions	Isolated fungi	Number of fungi	Frequency percentage
10^{-3}	*Aspergillus flavus*	14	32.5
	Rhodotorula	11	25.5
	Penicillium	9	20.9
	Candida spp.	9	20.9
	Chi-Square-χ^2	--	1.558 NS
10^{-4}	*Rhodotorula*	19	48.7
	Candida spp.	15	38.4
	Aspergillus flavus	5	12.8
	Chi-Square-χ^2	--	8.085*
10^{-5}	*Penicillium*	17	42.5
	Rhodotorula	12	30
	Candida spp.	11	27.5
	Chi-Square-χ^2	--	1.569 NS
10^{-6}	*Aspergillus flavus*	24	57.1
	Metarhizium anisopliae	10	23.8
	Trichoderma harzianum	8	19.4
	Chi-Square-χ^2	--	10.971**
Total: Chi-Square-χ^2 (P-value)		--	12.846** (0.00638)
(P≤0.05)* (P≤0.001)**			

Table 3: Dilution, Isolated Fungi, and Percentage Frequency in the 5 Kilo Area

Dilutions	Isolated fungi	Number of fungi	Frequency percentage
10^{-3}	0	0	0.00
	Chi-Square-χ^2	--	NS
10^{-4}	0	0	0.00
	Chi-Square-χ^2	--	NS
10^{-5}	*Aspergillus flavus*	18	78.2
	Penicillium	5	21.7
	Chi-Square-χ^2	--	7.347**
10^{-6}	*Aspergillus flavus*	11	45.8
	Penicillium	9	37.5
	Fusarium spp.	4	16.6
	Chi-Square-χ^2	--	3.285NS
Total: Chi-Square-χ^2 (P-value)		--	8.0179** (0.00895)
(P≤0.001) **			

Table 4: Dilution, Isolated Fungi, and Percentage Frequency in the Warar Area

Dilutions	Isolated fungi	Number of fungi	Frequency percentage
10^{-3}	0	0	0.00
	Chi-Square-χ^2	--	NS
10^{-4}	0	0	0.00
	Chi-Square-χ^2	--	NS
10^{-5}	*Aspergillus flavus*	4	57.1
	Fusarium spp.	3	42.8
	Chi-Square-χ^2	--	0.142 NS
10^{-6}	0	0	0.00
	Chi-Square-χ^2	--	NS
	Total: Chi-Square-χ^2 (P-value)	--	3.711 * (0.497)
(P≤0.05) *			

Table 5: Dilution, Isolated Fungi, and Percentage Frequency in the Soufia Area

Dilutions	Isolated fungi	Number of fungi	Frequency percentage
10^{-3}	0	0	0.00
	Chi-Square-χ^2	--	NS
10^{-4}	*Candida spp.*	24	31.1
	Rhodotorula	24	31.1
	Aspergillus flavus	15	19.5
	Trichoderma harzianum	9	11.6
	Rhizopus	5	6.5
	Chi-Square-χ^2	--	19.298 **
10^{-5}	*Candida spp.*	16	38
	Penicillium	10	23.8
	Rhodotorula	8	19
	Aspergillus flavus	8	19
	Chi-Square-χ^2	--	4.095 *
10^{-6}	*Penicillium*	15	37.5
	Aspergillus flavus	12	30
	Candida spp.	7	17.5
	Trichoderma harzianum	6	15
	Chi-Square-χ^2	--	5.400 *
Total: Chi-Square-χ^2 (P-value)		--	22.278 **
(P≤0.05) * (P≤0.001) **			

Table 6: Dilution, Isolated Fungi, and Percentage Frequency in the Officers' Quarter Area

Dilutions	Isolated fungi	Number of fungi	Frequency percentage
10^{-3}	0	0	0.00
	Chi-Square-χ^2	--	NS
10^{-4}	0	0	0.00
	Chi-Square-χ^2	--	NS
10^{-5}	*Aspergillus flavus*	18	75
	Penicillium	6	25
	Chi-Square-χ^2	--	6.143 **
10^{-6}	*Aspergillus flavus*	11	45.8
	Penicillium	9	37.5
	Fusarium spp.	2	8.3
	Rhizopus	2	8.3
	Chi-Square-χ^2	--	11.000 **
Total: Chi-Square-χ^2 (P-value)		--	13.549 ** (0.0006)
(P≤0.001)**			

(P≤0.05): Significant ** (P≤0.01): Highly significant NS: Not significan

Molecular Diagnosis of Fungal Isolates

Following the morphological and microscopic isolation and diagnosis of the fungal isolates under study, two different isolates were selected, with two replicates for each isolate, for the extraction and measurement of DNA concentration and purity using the Nanodrop device. The results revealed the possibility of extracting DNA from the fungal isolates with good concentrations and purity according to the equipment used. The purity ranged between 1.69 and 1.94, with concentrations ranging from 34.03 to 52.10 ng/ml, as shown in Table 7.

The Electric Migration of PCR Amplification Products.

The results revealed the amplification of the ITS gene located between rDNA genes using the forward and reverse primers ITS1 and ITS4 for two isolates, with two replicates for each isolate. The targeted ITS gene amplification was achieved through the polymerase chain reaction (PCR) technique using specific primers and electrophoresis of the amplification products. Bands appeared for all samples at approximately 550bp compared to the DNA ladder (1000 plus), indicating primer binding and amplification occurring in all samples, as shown in Figure (1).

Diagnosing isolates under study relies on the nucleotide base sequencing of the ITS gene:

After sending two different samples of DNA amplification products through the polymerase chain reaction (PCR) technique with the forward and reverse primers ITS1 and ITS4 to Macrogen Company in Korea to determine and identify the nucleotide base sequencing of the ITS gene, the sequences were analyzed using the BLAST program (Basic Local Alignment Search Tool), after being analyzed using the Chromas program and Mega-X program. Then, the results were compared with the data recorded in the National Center for Biotechnology Information (NCBI), belonging to the same globally registered strains (http://www.ncbi.nlm.nih.gov/). The following results were recorded in Table (8).

Table 7: Measurement of DNA Concentration and Purity for Fungal Isolates under Study Using the Nanodrop Device

260/280 purity	(ng/ml).Nucleic acid conc	Sample ID
1.71	**15.40**	**1**
1.69	**36.81**	**2**
1.83	**52.10**	**3**
1.94	**34.03**	**4**

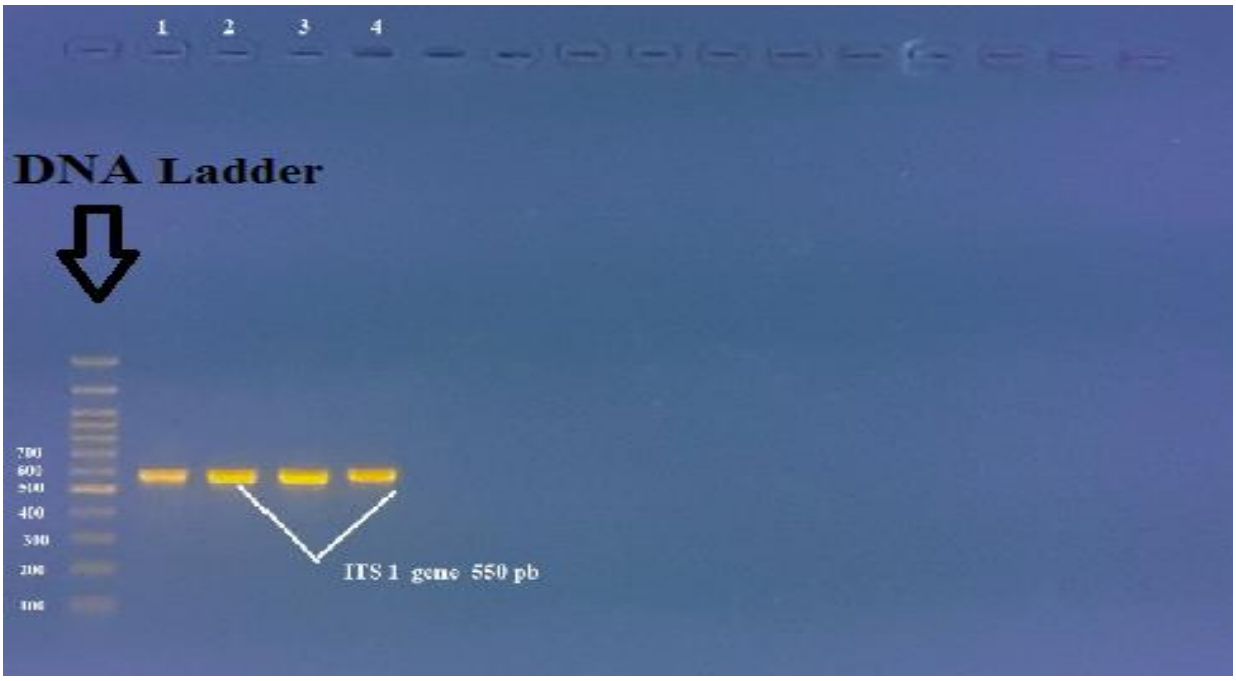

Figure (1) shows the electrophoresis of PCR amplification products using the primers ITS4 - ITS1 for four isolates on 1.5% agarose gel at 70 volts for one hour. Bands appeared at approximately 550 bp, compared to the DNA ladder (1000 plus).

Table 8: Global Isolates, Their Accession Numbers, and Country Names in NCBI Compared Using the BLAST Program Showing the Type of Variation in Bases Along with the Percentage of Matching with the Isolates under Study.

No	Type of substitution	Location	Nucleotide	Sequence ID with compare	Country	Source	Identities
1	-	-	-	ID: MZ356389.1	India	*Metarhizium anisopliae*	100%
2	Transvertion	239	C\A	ID: MZ130515.1	India	*Trichoderma harzianum*	98.58
	-	255	-\G				

Analysis of the sequences of isolate number 1 under study:

Alignment results of isolate sequences (1):

The alignment results and analysis of the nucleotide base sequencing of isolate number (1) after comparing them with the sequences registered in NCBI indicate their match with sequences registered in India and belonging to the species *Metarhizium anisopliae*, with the identification number ID: MZ356389.1 in NCBI, with a matching percentage of 100%, as shown in Figure (2).

The taxonomic classification of isolate number (1) as indicated by the NCBI matching result is:Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreomycetidae; Hypocreales; Clavicipitaceae; *Metarhizium*.

The genetic relationship between the studied isolate and globally recorded:

isolates are represented in Figure (3) Phylogenetic Tree for isolate number (1). The isolate labeled as M. anisopliae Safana is highlighted in red, along with globally recorded isolates, as determined by the BLAST program.

The nitrogenous base composition percentages in the ITS gene of isolate number (1) :

The shape (4) shows the ratios of the four nitrogenous bases in the ITS gene of the isolated strain under study. The results indicate that the length of the globally analyzed and compared segment is (504 bp), distributed among the four nitrogenous bases as follows:

| A(23.21% 117) | C(28.37% 143) | G(30.36% 153) | T(18.06% 91|

Analysis of sample number (2):

Alignment results of isolate (2) sequences.

The alignment results and analysis of the nucleotide sequence of isolate number (2), after comparison with sequences recorded in NCBI, reveal that they match with sequences recorded in India for the species *Trichoderma harzianum*, with the accession number ID: MZ130515.1 in NCBI, with a matching percentage of 98.58%.

Additionally, Figure (5) illustrates two variations (mutations). A mutation was observed at position 239, where the cytosine base (C) was recorded in the sample under study instead of the adenine base (A). This variation (mutation) was identified as a non-synonymous substitution type called Transversion. Another mutation was observed at position (255) in the sample recorded in NCBI, where the guanine base (G) was encountered, while it was not determined in the sequences under study.

Taxonomic classification of isolate number (2):

The matching result in NCBI revealed the taxonomic classification of the sequences of isolate number 2 as follows:

Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Sordariomycetes; Hypocreomycetidae; Hypocreales; Hypocreaceae; *Trichoderma*.

The genetic relationship between the isolate under study and globally recorded isolates:

Figure (6) illustrates the Phylogenetic Tree of isolate number 2, with the strains marked in red under the name *Trichoderma harzianum*, alongside globally recorded isolates, as determined by the BLAST program.

The percentages of nitrogenous bases in the ITS gene for isolate number (2):

Figure (7) illustrates the proportions of the four nitrogenous bases in the ITS gene for the isolate under study. The results indicate that the length of the segment analyzed and globally compared is 140 bp, distributed among the four nitrogenous bases as follows:

These results are consistent with Al-Nadawi, (27) and Shanker *et al.*, (28), who found that the ITS region showed diagnostic potential for the fungus *M. anisopliae*, as well as Francis (29) and Manchegowda, who were able to diagnose two

species of *Metarhizium*, *M. guizhouense* and *M. pingshaense*, using the ITS region. They emphasized the necessity and importance of molecular diagnosis in accurately distinguishing between species and strains. Similarly, Mazrou *et al.* (30) successfully diagnosed *T. harzianum* using PCR and the primers ITS1 and ITS4, with a sequence match percentage of 98% among tested isolates.

Additionally, Stummer *et al.* (31) identified three species of *Trichoderma* - *T. harzianum*, *T. afroharzianum*, and *T. gamsii* - through ITS gene amplification using PCR. Mahmoud *et al.* (32) molecularly diagnosed nine isolates of *T. harzianum* using PCR. In a study by Gezgin *et al.* (33), T*richoderma* fungi were diagnosed using two different genetic regions (ITS and EF) for molecular identification, with amplification of these genes through PCR.

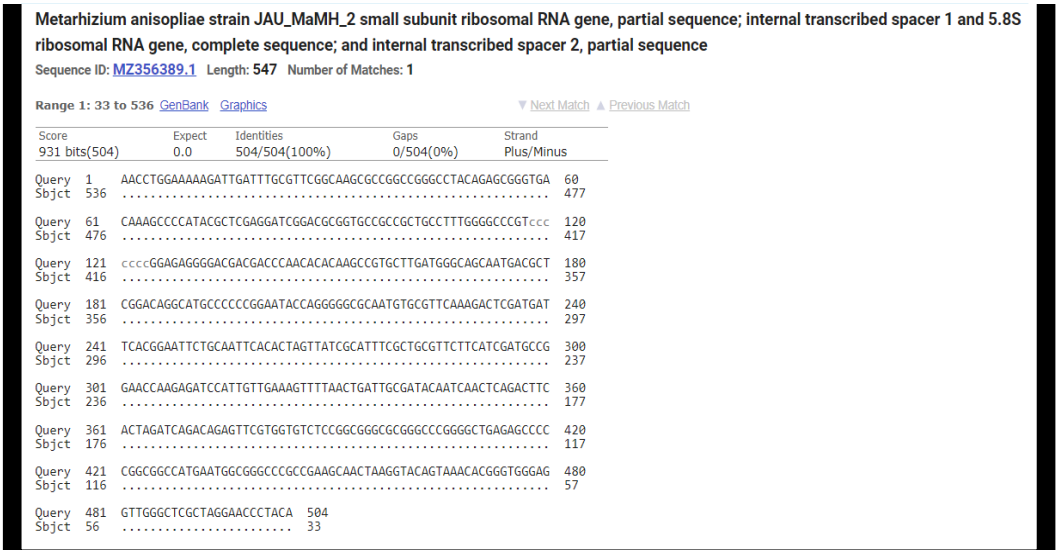

Figure (2) Nitrogenous Base Sequences in BLAST Program between the studied sequence and the top match isolate of *Metarhizium anisopliae* recorded in India under accession ID: MZ356389.1 with 100% matching percentage.

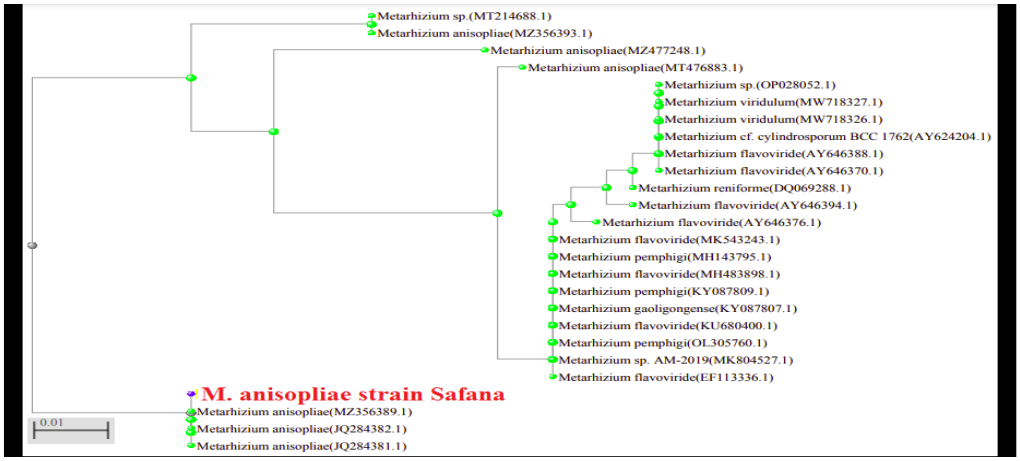

Figure (3) Displays the Phylogenetic Tree illustrating the genetic relationship of isolate number (1). The isolate labeled M. anisopliae Safana is highlighted in red, alongside globally recorded isolates identified through the BLAST program.

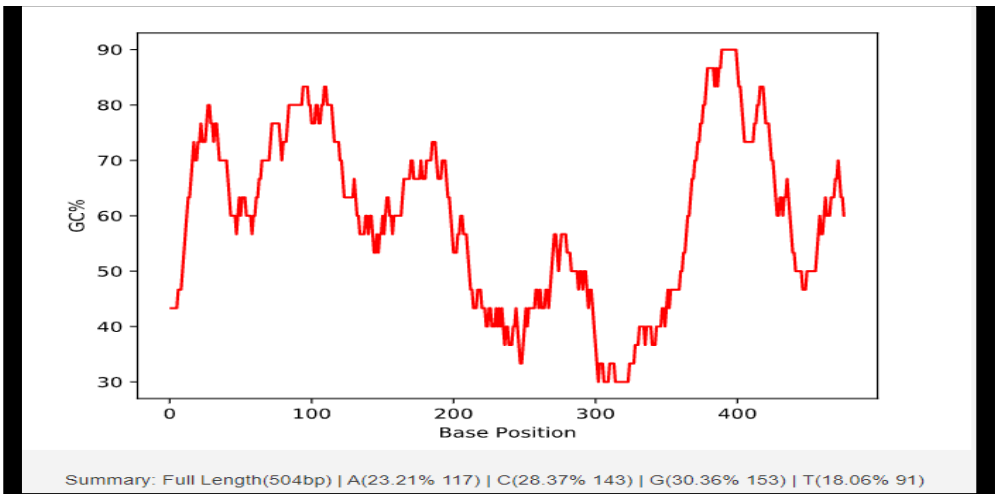

Figure 4 illustrates the proportions of the four nitrogenous bases in the ITS gene of Isolate (1).

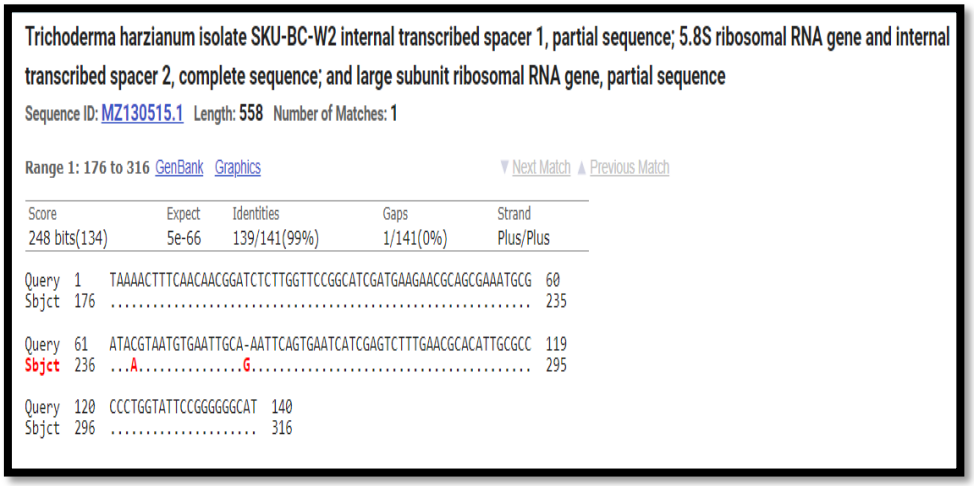

Figure (5) Displays the Nucleotide sequence alignment in the BLAST program between the sequences under study and the top matching isolate of *Trichoderma harzianum* recorded in India under the accession number ID: MZ130515.1, with a matching percentage of 98.58%.

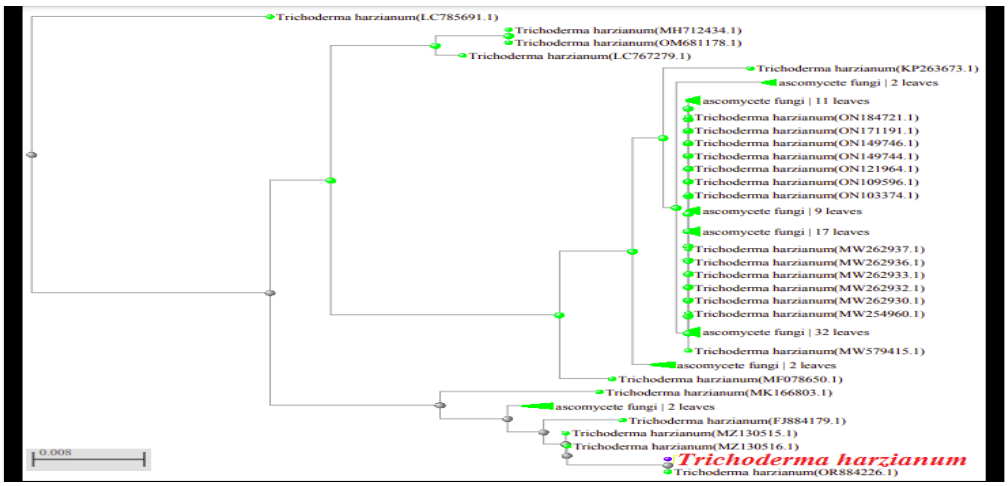

Figure 6: Depicts the Phylogenetic Tree of isolate number (2), with *Trichoderma harzianum* highlighted in red, along with globally recorded isolates as determined through the BLAST program.

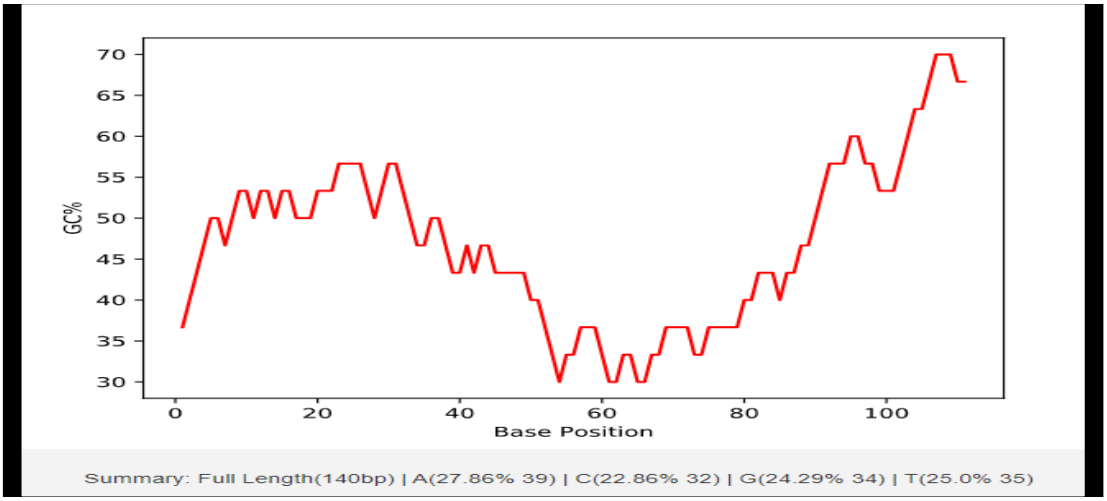

Figure (7) Depicts the relative proportions of the four nitrogenous bases in the ITS gene for isolate number 2.

Conclusion:

Non-culture-based methods, such as PCR, allow for rapid detection of infections, facilitating targeted treatment towards pathogenic and effective species and identifying genetic markers associated with antifungal resistance. Molecular amplification techniques enable rapid and sensitive detection by directly detecting small amounts of fungal DNA in clinical samples without the need for prior culture, making these tests attractive for early disease diagnosis. This region contains multiple synonymous repetitions of ribosomal DNA, making it very useful in species identification and hence proposed as a standard marker in encoding fungal DNA barcodes.

Conflicts of interest

"There are no conflicts to declare".

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