Journal of Current Medical Research and Opinion
Received 29-04-2024 | Revised 30-04-2024 | Accepted 21-05-2024 | Published Online 23-05-2024

DOI: https://doi.org/10.52845/CMRO/2024/7-5-27 ISSN (O) 2589-8779 | (P) 2589-8760

CMRO 07 (05), 2589-2596 (2024)

Original Research

Biodegradation of Paper Waste by *Fusarium* spp.

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Abstract:

This study was conducted to detect and identify most important of Fusarium isolates that can degrades waste paper in environment, detection their ability to production Cellulase enzyme who, is responsible in biodegradation process. Fielded inspection and isolation and identification process revealed three species *Fusarium oxysporum*, *F. solani*, and *F. pllyphialidicum* were the most important in waste biodegradation, while F. roseum was the less in biodegradation ability. Grown tested on solid waste paper showed *Fusarium oxysporum* was the most capable of growing in mediumin ratio near by 78 % than followed by *F. solani* in 63 % and *F. pllyphialidicum* was 50 % comperin with *F. roseum* which was the less one in capability in growing on solid waste paper medium.

The growth results of the isolates on liquid media were confermed with the results of solid media, as the fungus *F. oxysporum* ranked first in growth based on the weight of the bio mass with significant differences which was 255.1 mg compering with the latest isolate *F. roseum* 119.6 mg, while *F. solani* was the second one with significant differences reached to223.4 mg with last one, also *F. pllyphialidicum* has a 189.2 gm significant differences with *F. roseum*. Chromogenic method by using Gongo red stain was apply to detect efficiency of *Fusarium* species to producing cellulose enzyme to degradation waste paper considering clear zone as indicator to cellulose analysis, *F. oxysporum* has a significant impact with length of clear zone was 31 mm compering with the latest isolate *F. roseum* 19 mm, then *F. solani* was the second one with significant differences was 26 mm and the third was *F. pllyphialidicum* compering with *F. roseum* 19 mm.

Keywords: Fusarium, Paper Waste, Biodegradation

Introduction:

Paper products are one of the most important inputs in the various production processes (Science Direct. 2018). The most common manufactured products that are consumed on a daily and continuous basis are often packaged and wrapped using paper products, whether these products are medical, food, agricultural, industrial, and even electronic products, as well as About the widespread and major use of paper products in administrative and official correspondence of various governmental institutions and academic and educational institutions, essentially, in all

civilized human societies throughout the world (Iqtidar et al. 2019) The excessive use of paper products in these areas has generated environmental pressure that may have significant negative consequences that threaten the environmental and biological balance (Beckline et al. 2016).

Researchers have noticed the importance of recycling and biodegradation of paper waste since a long time. Paper waste has been used as a source of energy production by using paper waste as fuel to generate the heat needed to operate electrical power plants, but this procedure has major negative environmental consequences and has a negative impact on the ozone layer through harmful thermal emissions and increasing the concentration of CO2 gas to unacceptable levels (Ahmed et al. 2018).

Biotechnology in the recycling and biodegradation of paper waste is one of the most important effective and efficient methods through which important biological tools can be obtained in the field of energy production and in the industrial sector, such as the production of the important ethanol (Ozola et al. 2019). In addition to being environmentally friendly, fungi are among the widespread microorganisms in nature and have very important environmental effects in recycling paper waste naturally in the environment through the effective and efficient enzymatic system that they are characterized by (Mathivanan et al. 2017). Fungi have been used in many food and fermentation industries in abundance. Its production of biological enzymes (Shah et al. 2022).

Fusarium is one of the most famous fungi known for its abundant production of extracellular enzymes, such as cellulase and pectinase, among the most famous enzymes produced by fungi, as they are the bioreactor for decomposing plant waste in forests and agricultural fields throughout the world (Kwon et al. 2017). These enzymes are also considered among the most important weapons of parasitic fungi. Plant pathogen (Sharafaddin et al. 2019).

Paper is usually manufactured as a thin sheet from cellulose pulp derived from wood and other lingo cellulosic materials such as cotton, rice or wheat straw (Latha et al. 2018), this paper was conducted for screening and detection ability of studied isolates in producing cellulase and pectinase enzymes in laboratory conditions.

Materials and Methods:

1- Collecting samples, isolating and identification of fungi:

Sites were chosen from different areas of Babil Governorate that are famous for being sites for discarding paper waste. Pieces of discarded paper waste mixed with soil were collected and kept in paper envelopes and transported to the fungi laboratory at the College of Biotechnology and preserved. In the refrigerator until performing the following tests.

Distributed 4 square pieces, 1 cm long, of paper waste inside Petri dishes and poured PDA medium on them. The dishes were incubated at 28 Co for 5 days, then the fungal colonies were purified in individual dishes.

The fungi were diagnosed in the Department of Biotechnology using the following keys Hoching ,pitt 1997 ,Booth. 1977, Domsch, 1981, Ellis. 1971

2- The ability of isolates to grown on solid paper media

Pieces of paper used for writing were prepared, length 1 x 1 cm, mixed with distilled water at a concentration of 1%, the mixture was placed in a blender for 5 minutes, then agar- agar was added to the medium in order to test the ability of the fungal isolates on a culture medium supported by paper waste, and to sterilize the medium with an autoclave device.

Petri dishes were prepared with medium supplemented with paper waste, then inoculated with 0.5 cm discs from fresh cultures of the studied isolates and incubated for 5 days at 28 C^{O} for 5 days, and then the diameter growth of the developing colonies were measured.

3- The ability of isolates to grown on liquid paper media

The same previous medium was used, but in liquid form without adding agar, to measure the ability of the studied fungal isolates to grow on a nutrient medium supplemented with leaf waste by measuring the live mass of the growing colonies. After performing the same previous treatments, the treatments were incubated for 28 C^{O} For 5 days, then the biomass was estimated based on the weight of the colony mass

4- Detection cellulose efficiency on Gongo red medium

In order to detect the efficiency of the cellulase enzyme in degradation the paper waste supplemented with medium, a solid culture medium was prepared supplemented with 1% of Carboxymtyl cellulose (CMC), 1% of paper waste and by adding a reagent of Gonco red dye at a rate of 0.1%. The dishes prepared with the mentioned medium were inoculated with an inoculum for the studied isolates, then incubated 28 C^{o} for a period of 5 days, after which the diameter of clean zone surrounding of fungal inoculum were measured.

Results and Discussion:

1- Fungi identification:

The results of isolating and diagnosing fungi from the examined soil samples showed the isolation of a wide spectrum of fungal isolates, and the focus was on four isolates from four species belonging to the genus Fusarium for the rest of the laboratory experiments.

Figuer 1 clearly reveal the isolate were: *Fusarium oxysporum*, *Fusarium solani*, *Fusarium polyphialidicum* and *Fusarium roseum*

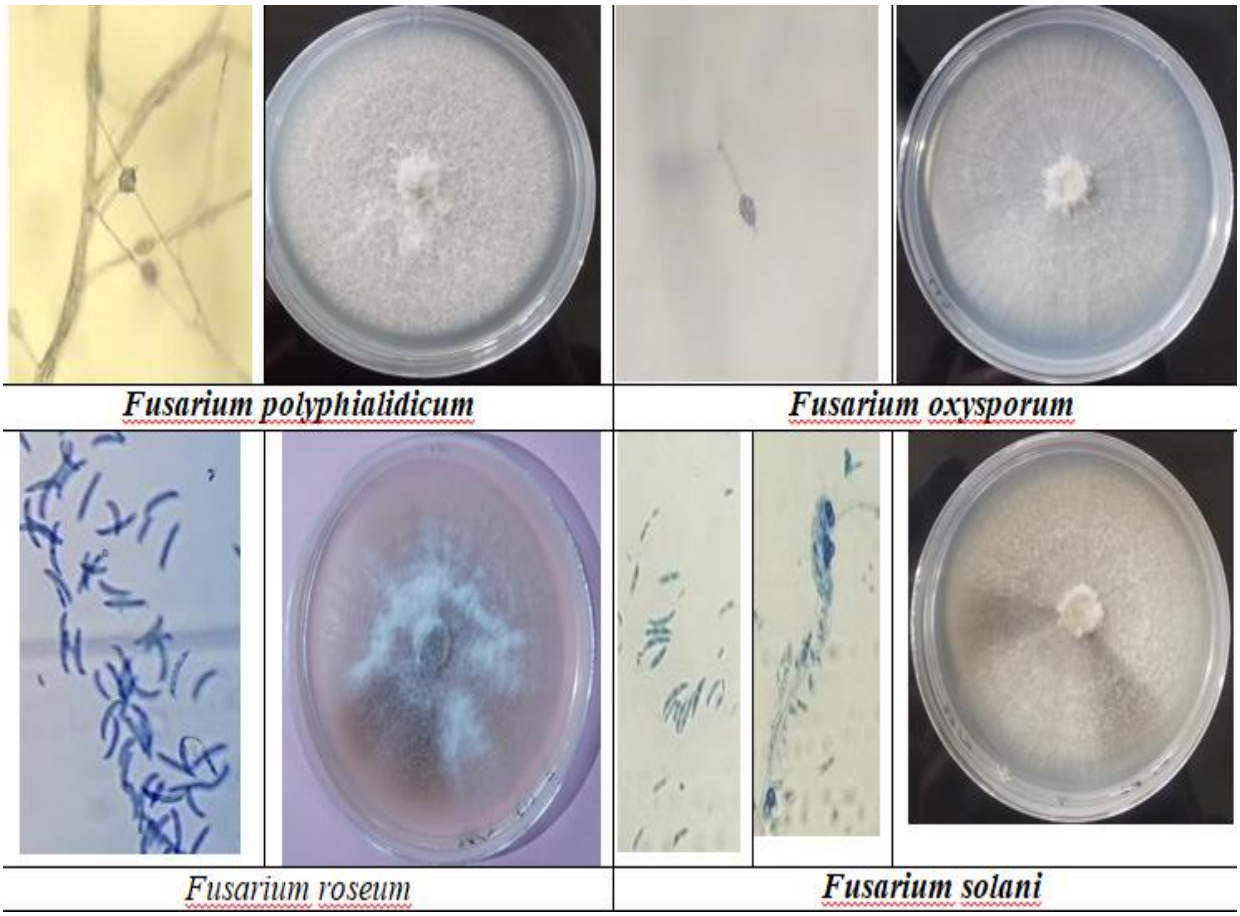


Figure1: Macro- micro characteristics of studded isolates

2- The ability of isolates to grown on solid paper media:

The results of growing the studied isolates on a weak solid medium supplemented with 1% paper waste indicate variation in the ability of these fungal isolates to grow, as shown in Table 1.

Table 1. Growth radius of fungi colonies on weak solid medium supplemented with 1% paper waste

No.	Isolate	Radius of colon\ mm
1	**Fusarium oxysporum (F 1)**	**68**
2	**Fusarium solani (F 2)**	**62**
3	**Fusarium polyphialidicum (F 3)**	**57**
4	**Fusarium roseum (F 4)**	**38**
***P*≤ 0.05 (L.S.D= 5. 196)**		

The results clearly indicate that the fungus *Fusarium oxysporum* was significantly superior to the fungus *Fusarium roseum* in its ability to grow on a solid medium supplemented with 1% of paper culture, followed by the fungus *Fusarium solani* in second place, then the fungus *Fusarium polyphaidicum*, while the fungus *Fusarium roseum* ranked last in terms of Its ability to grow on a solid medium supported by 1% of paper waste

3- The ability of isolates to grown on liquid paper media

The results of the growth of the study isolates on a liquid medium supplemented with 1% paper waste to figure out their ability to grow, and the degradate the paper waste showed conformed results from the previous test on the soild medium, by measuring the weight of the bio mass, estimated in milligrams, table 2.

Table 2. the biomass of tested isolates grown on liquid medium supplemented with 1% cellulosic paper waste evaluated by milligram.

No.	Isolate	Weight of biomass\ mg
1	***Fusarium oxysporum* (F 1)**	**255.1**
2	***Fusarium solani* (F 2)**	**233.4**
3	***Fusarium polyphialidicum* (F 3)**	**221.2**
4	***Fusarium roseum* (F 4)**	**102.6**
***P* ≤ 0.05 (L.S.D=111.0827)**		

The results clearly indicate that the fungus *Fusarium oxysporum* was significantly superior to the fungus *Fusarium roseum* in its ability to grow on a liquid medium supplemented with 1% of paper culture, followed by the fungus *Fusarium solani* in second place, then the fungus *Fusarium polyphaidicum*, while the fungus *Fusarium roseum* ranked last in terms of Its ability to grow on a liquid medium supplemented with 1% of paper waste, Figure 2.

Figure 2: Biomass of studded isolates grown on 1% paper

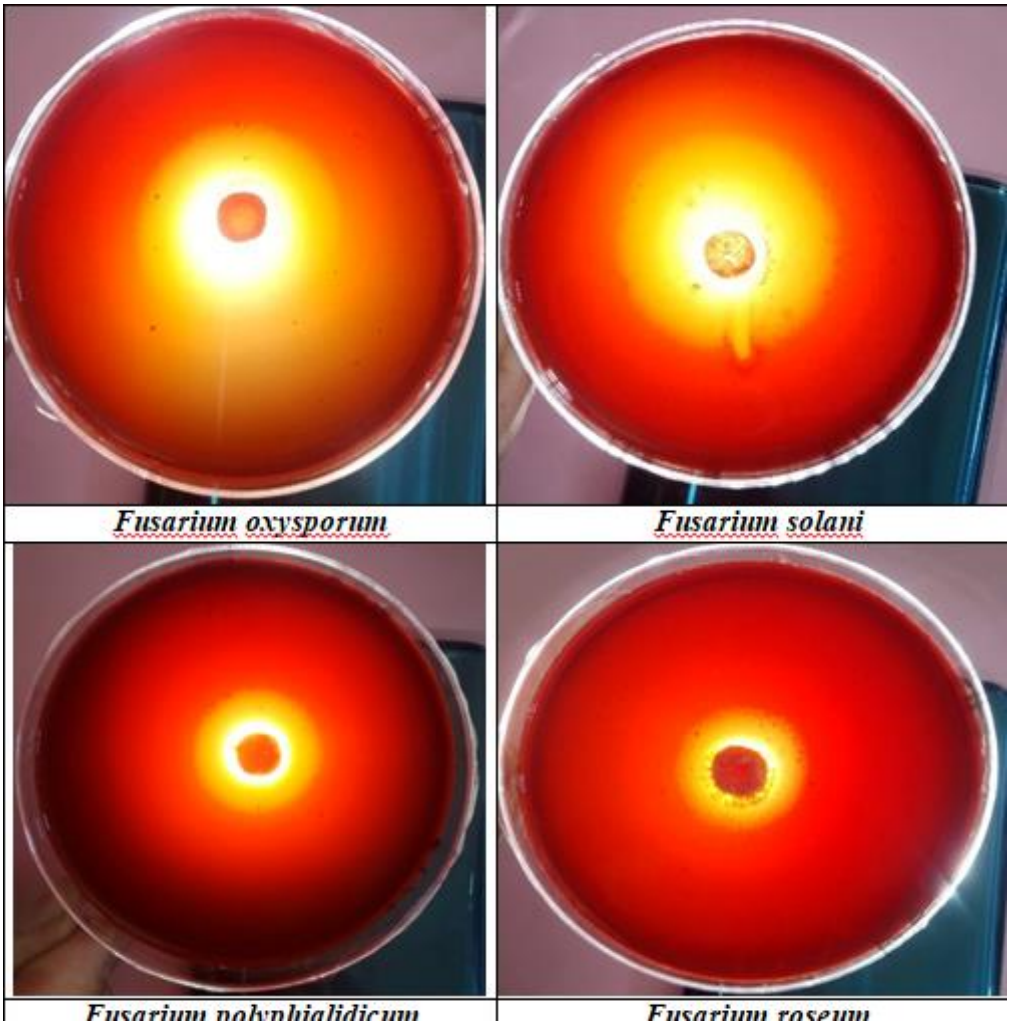
4- Detection cellulose efficiency on Gongo red media

The results listed in Table 3 indicate the length of the diameter of the clear zone resulting from the degradtion of carboxymethylcellulose (CMC) resulting from the ability of the studied isolates to produce the cellulase enzyme that breaks down cellulase, which is the main component of paper waste. It was detected with the help of Gongo Red stain measured by mm figure 3.

Tale 3: Detection of Celluloase enzyme by length of clear zone degradation of CMC using Gongo red stain

No.	Isolate	Weight of biomass\ m
1	***Fusarium oxysporum* (F 1)**	**31**
2	***Fusarium solani* (F 2)**	**28**
3	***Fusarium polyphialidicum* (F 3)**	**26**
4	***Fusarium roseum* (F 4)**	**19**
***P* ≤ 0.05 (L.S.D= 5.284)**		

The results clearly indicate that the fungus *Fusarium oxysporum* was significantly superior to the fungus *Fusarium roseum* in terms of the effectiveness of the cellulase enzyme in biodegrading leafy tissue, followed by the fungus *Fusarium solani* in second place, then the fungus *Fusarium polyphaidicum*, while the fungus *Fusarium rosem* ranked last in terms of the efficiency of the cellulase enzyme in the biodegradation of paper waste, Figure 3.



Figuer 3: Detection Cellulase enzyme efficiency by clear zone shown due to analyzing CMC using Gongo red stain

Discussion:

The genus *Fusarium* is one of the most common and widespread worldwide, can be habitat in food staff like cereals (Al-Defiery and Merjan. 2015), fungi in nature and succeeds in existing in various environments. It can be present in agricultural soil and parasitize on the roots of wild plants and agricultural crops (Merjan ei al. 2019, Otibi et al. 2023). It can live in the soil of waste dumps in a discarded form, and then it can It plays an important role in the biodegradation processes of organic waste, especially cellulosic paper waste (Obire et al .2002, Merjan et al . 2020). In addition to its high ability to produce toxic metabolic compounds associated with cancer in humans(Merjan et al. 2023)

The culture medium supplemented with organic materials in low concentrations is considered a poor nutrient medium, so the ability of fungal isolates to grow and bacterium on it varies (Srivastava et al. 2011).

Fusarium has a wide spectrum system of extracellular enzymes through which they can break down organic materials, analyze them, and then feed on them (Kwon et al.2007). Fungi are among the most efficient microorganisms that produce extracellular enzymes (Bakri et al. 2014).

The cellulase enzyme produced from fungal cells breaks down the cellulose present in the fungal feeding medium and converts it into structurally simpler materials in order to provide the necessary and essential carbon element in feeding the Fusarium fungal isolates (Dutta et al. 2018).

Fungi, especially Fusarium, contribute significantly to achieving environmental balance through their high ability to break down organic waste, including paper waste, the long period of their stay in the environment leads to a major and

dangerous imbalance in the environmental balance (Otibi et al. 2023).

Conclusion:

Waste paper is one of phenomena pollutant that pollute Babylon environment and must had a lot of attention. *Fusarium* spp are great role in eco-friendly of biodegradation og waste like paper with. Some Fusarium species have active cellulolytic set enzyme to breakdown organic waste paper

Recommendation:

Forwarded towered treating pollution problem especially paper waste in Babylon govern ate. Follow eco- friendly techniques like biodegradation of paper waste by using *Fusarium* spp strains. Their a huge chance for using waste paper with help of *Fusarium* spp to produce bio fuel like ethanol. Contention with research to eradicate waste paper by biodegradation and using the product in industry field.

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