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Development of Mycological Medium using Tomato Juice Extract as Principal Base

Okoye Rosemary [1]* and Abba Oluchukwu[2]

[1,2]Department of Microbiology, Federal University Gusau, 860242, Zamfara State, Nigeria

*Correspondence author: rosemaryokoye@fugusau.edu.ng, +234 7032565178

Abstract

This project focused on developing mycological media using the primary base of Tomato Juice Extract (TJA). Tomatoes contain a rich mix of vitamins, minerals, and organic acids, creating an ideal environment for cultivating various fungi. Various concentrations of Tomato Juice Agar (TJA) and Potato Dextrose Agar (PDA) were prepared (200 mL, 300 mL, and 400 mL). TJA was created by combining tomato filtrate with different sugar concentrations (10g, 15g, 20g), keeping agar agar powder constant at 20 g. Samples of soil from refuse dumpsite and spoiled bread were inoculated onto the prepared TJA and PDA (control). The total fungal counts, isolation, and identification were determined using PDA and TJA. Microscopic analyses, serial dilutions, and colony characterization provided a detailed assessment. TJA exhibited total fungal counts of 2-3 propagules/g for refuse dumpsite soil and 3-4 propagules/ml for spoiled bread. PDA yielded counts of 3-6 propagules/g for refuse dumpsite soil and 3-5 propagules/ml for spoiled bread. Various fungi, including Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Rhizopus stolonifer, Penicillium. digitanum, and Fusarium oxyporum were identified. TJA showed selective preferences for Aspergillus niger and Rhizopus stolonifer. This study successfully developed mycological media with tomato juice extract, yielding promising fungal cultivation results. Different TJA concentrations influenced fungal growth, thus emphasizing the importance of selecting the appropriate medium. The absence of growth of some of the test fungal isolates on TJA and exclusive isolation of Fusarium oxysporum on PDA shows medium-specific preferences. Tomato juice extract aligns with sustainability and cost-effectiveness, offering an alternative to traditional components. Valuable insights into concentration-dependent fungal growth guide future experimentation and optimization. Tomato juice extract as a mycological medium base introduces a sustainable alternative with unique nutritional profiles. Concentration-dependent fungal preferences revealed the significance of medium optimization. These findings encourage the use of natural sources for more sustainable microbiological practices. While laying innovative mycological media groundwork, further research is needed to optimize tomato juice agar fully. Mycological media development with tomato juice extract offers practical benefits in cost-effectiveness and sustainability.

Keywords: Mycology, Tomatoes, Juice extract and Medium

INTRODUCTION

Mycological media have evolved, serving as essential tools for studying fungi and bacteria. The foundational concept involves creating basal media to which antifungal agents can be added, rendering them selective for isolating and cultivating fungi (Kibby and Geoffrey, 2023). Fungal Agar with a low pH is typically employed for saprophytic fungi, while Mycological Agar is preferred when working with pathogenic fungi. Earlier media for fungi often relied on an acidic pH to create an environment less suitable for bacterial growth, emphasizing the historical importance of pH manipulation in mycological media (Keegan *et al.*, 2013).

Microorganisms, including fungi, require specific nutrients, energy sources, and certain environmental conditions for growth and reproduction. In their natural habitat, microbes adapt to conditions suitable for their needs. However, these requirements must be met in a laboratory setting through culture media. A culture medium is essentially an aqueous solution to which all necessary nutrients have been added. This nutrient-rich environment

supports the growth of microorganisms and serves as a foundation for various types of media, depending on the combination of nutrients (Keegan *et al.*, 2013).

Protein hydrolysates are common components in complex media, often derived through enzymatic digestion or acid hydrolysis of natural products such as animal tissues, milk, plants, or microbial cultures. Protein hydrolysates, also known as peptones, serve as excellent natural sources of amino acids, peptides, and proteins crucial for microbial growth. Sterility is paramount in microbiological media, and proper sterilization techniques involving heat, chemicals, radiation, and filtration ensure the elimination of all microorganisms, including spores (David *et al.*, 2015).

The choice of tomato juice extract as the principal base for mycological media introduces an innovative and potentially advantageous approach. Tomato juice is rich in nutrients, vitamins, and antioxidants, which may contribute to the growth and development of fungi. The organic compounds in tomato juice, such as sugars, organic acids, and enzymes, could offer a unique nutritional profile for fungal cultures. This choice aligns with the broader understanding that the composition of the medium significantly influences microbial growth and behavior (David *et al.*, 2015).

Furthermore, using tomato juice extract aligns with exploring natural sources for mycological media components. Previous studies have highlighted the potential of plant extracts and organic compounds to promote microbial growth (Laesia and Petersen, 2016). Incorporating tomato juice extract into mycological media could offer a sustainable and cost-effective alternative to traditional media components. This led to this study, which focuses on the innovative development of mycological media, utilizing tomato juice extract as the principal base.

MATERIALS AND METHODS

Collection of Samples

Two samples were procured: refuse dumpsite soil and spoiled bread. The soil was aseptically collected from the Tudun Wada area using a sterile soil auger and placed in a sterile container. Spoilt bread was purchased from Unguwar Dallatu. Fresh tomatoes for the medium were acquired from the Tudun Wada market. The soil sample was transported in an ice box and analyzed within 24 hours, while the bread was stored in a polyethylene bag for 7 days before analysis.

Preparation of Samples

The refuse dumpsite soil samples were air-dried for one week, ground, sieved through a 2mm stainless sieve, and stored in a labeled plastic container. The bread sample was similarly air-dried, ground, and stored in a plastic container until analysis.

Preparation of Media

The potato dextrose agar (PDA) was weighed in a different concentration of 200, 300, and 400ml, prepared according to manufactured instructions, autoclaved at 121°C for 15 minutes, and poured into the Petri dishes allowed to cool and solidify. This was used to compare the growth of the formulated media.

Formulation of Tomato Juice Agar

Tomato juice agar (TJA) was aseptically prepared by heating 200 mL of distilled water at 90°C, and 20g of agar-agar was dissolved. Fresh tomatoes were washed and blended, and 200 mL of juice was mixed with agar. To this, 10g of glucose and streptomycin were added to inhibit bacterial growth. The pH was measured before and after autoclaving at 121°C for 15 minutes. After cooling, the medium was poured into sterile Petri dishes and allowed to solidify.

In another experiment, TJA was prepared with varying volumes of tomato juice (200 mL, 300 mL, 400 mL) and glucose concentrations (10g, 15g, 20g), with a constant 20g agar. The TJA plates were inoculated and incubated at 25°C for 3-5 days following Aigbogu *et al*. (2018).

Determination of Mycological Properties

Serial Dilution

A five-fold serial dilution was performed for the refuse dumpsite soil and spoiled bread samples. One gram of each sample was inoculated into 9 mL of sterile distilled water. Then, 1 mL of this homogenized sample was transferred to a second tube with 9 mL of sterile distilled water. This process was repeated until the fifth dilution. The sample from the third dilution was selected for microbial analysis.

Fungal Isolation

Total Fungal Count

Different concentrations (200 mL, 300 mL, 400 mL) of potato dextrose agar (PDA) and tomato juice agar (TJA) with streptomycin were prepared. Using the spread plate method, 0.1 mL of the third dilution (10^3) from the five-fold serial dilution was evenly spread on PDA and TJA plates. Plates were incubated inverted at 37°C for 5 days. Fungal colonies were counted, sub-cultured, and stored on sterile PDA and TJA agar slants for further analysis and identification (Chessbrough, 2006).

Characterization and Identification of Fungal Isolates

The molds were characterized and identified using the lactophenol cotton blue test and microscopic examination. The identity of each fungus was confirmed using a mycological atlas.

Slide Culture Technique

A small section of the aerial mycelia was introduced onto slides with prepared potato dextrose agar and tomato juice agar using a sterile inoculating needle. The slide underwent incubation at room temperature for twenty-four hours, following which it was observed under the microscope using a 10× and 40× objective lens.

Microscopic Analysis

Lactophenol cotton blue solution was employed for the microscopic analysis. A sterile straight wire loop retrieved a small portion of the fungal colony from the subculture PDA and TJA plates. Two drops of lactophenol cotton blue solution were dispensed onto a clean slide. The isolate was collected with a sterile syringe and transferred onto a glass slide. Subsequently, a sterile coverslip was placed over the stain, and the sample was examined under a microscope using a 40× objective lens.

Colony Morphology and Cellular Morphology

The colony morphology was determined based on the color, elevation, margin, form, and shape, while the cellular morphology was determined based on the cell's shape and arrangement.

RESULTS

The results of the total fungal count analysis for Tomato Juice Agar (TJA) for refuse dumpsite soil and spoiled bread are detailed in Table 1. The results reveal a range of 2-3 cfu/ml across the concentration range of 200 to 400 ml for refuse dumpsite soil. Conversely, the spoiled bread exhibits a range of 3-4 cfu/ml across the various concentrations.

Table 1: Total fungal count for Tomato juice agar (TJA) for refuse dumpsite soil and spoiled bread.

Concentration/ml	Refuse dumpsite soil (cfu/ml)	Spoiled bread (cfu/ml
200	3	4
300	2	3
400	2	3

The results of the total mold Count on Potato Dextrose Agar (PDA) for refuse dumpsite soil and spoiled bread are illustrated in Table 2. The total fungal count on PDA for refuse dumpsite soil falls within the range of 3-6 CFU/ml across different concentrations, as evidenced by the results obtained for each concentration during the analysis. Conversely, the total fungal count on PDA is within the range of 3-5 cfu/ml for spoiled bread.

Table 2: Total fungal count for Potatoe dextrose agar (PDA) for refuse dumpsite soil and spoiled bread.

Concentration/ml	Refuse dumpsite soil (cfu/ml)	Spoiled bread (cfu/ml
200	6	5
300	4	3
400	3	3

The colony and microscopic characteristics of mold isolate from refuse dumpsite soil and spoiled bread are presented in Table 3. The mold isolated where *Aspergillus Flavus, Aspergillus Fumigatus, Aspergillus Niger, Penicillium digitanum, Fusarium oxyporum, and Rhizopus. Stolenifer.*

Table 3: Colony and Microscopic Characteristics of Mold Isolated from Refuse Dumpsite Soil and Spoiled Bread.

Isolate	Colonial characteristic	Microscopic characteristic	Organisms
1	Spreading yellow-Green colonies are granulars, flat, mature vesicles over their entire surface.	Septate hyphae, individual conidiophores, carry conidia at the top head.	*Aspergillus flavus*
2	Uniseriate aspergilla with columnar condial heads in a shade of dark green and flask-shaped.	Septate hypae individual conidiophores with end bulb.	*Aspergillus Fumigatus*
3	Uniseriate or biseriate conidial head spherical, smooth, and black coloured.	Septate hyphae individual conidiophores with a long tip	*Aspergillus Niger*
4	Green consists of dense felt of conidio penicillum phores.	Hyphomycete, flask-shaped phialides arranged in group.	*Penicillium digitatum*
5	White become orange with discrete orange sporodochia present in some strain.	Non Septate, ellipsoidal to cylindrical	*Fusarium oxysporum*
6	White cottony at first becoming brownish grey to blackish grey depending on the amount of spurulation.	Globase, subglobase oval collapsing to an unbrella after spore release	*Rhizopus stolonifer*

The occurrence of mold isolates in different concentrations of Tomato Juice Agar (TJA) and Potato Dextrose Agar (PDA) from refuse dumpsite soil is shown in Table 4. *Rhizopus stolonifer* and *Aspergillus niger* were isolated in concentrations ranging from 200 to 400 ml in Tomato Juice Agar (TJA). *Aspergillus flavus* was isolated solely in the 200 ml concentration, while *Aspergillus fumigatus, Penicillium digitatum*, and *Fusarium oxysporum* were not detected in the various concentrations of Tomato Juice Agar (TJA). On the contrary, *Rhizopus stolonifer*, *Penicillium digitatum*, and *Fusarium oxysporum* were identified in the diverse concentrations of Potato Dextrose Agar (PDA). *Aspergillus flavus, Aspergillus fumigatus, and Aspergillus niger* were exclusively isolated in the 200 ml concentration of the Potato Dextrose Agar (PDA).

Table 4. Occurrence of fungal isolate in different concentrations (TJA) and (PDA) for refuse dumpsite soil

Isolates	Organisms	TJA concentrations/ml			PDA Concentrations/ml		
		200	300	400	200	300	400
1	*Aspergillus flavus*	+	-	-	+	-	-
2	*Aspergillus Fumigatus*	-	-	-	+	-	-
3	*Aspergillus Niger*	+	+	+	+	+	-
4	*Penicillium digitatum*	-	-	-	+	+	+
5	*Fusarium oxysporum*	-	-	-	+	+	+
6	*Rhizopus stolonifer*	+	+	+	+	+	+

The occurrence of mold isolates in various concentrations of Tomato Juice Agar (TJA) and Potato Dextrose Agar (PDA) for spoiled bread is presented in Table 5. *Aspergillus flavus* was isolated in the 200 ml of Tomato Juice Agar (TJA). On the other hand, *Aspergillus fumigatus* and *Aspergillus niger* were consistently isolated across all concentrations of both TJA and Potato

Dextrose Agar (PDA). *Rhizopus stolonifer* was isolated in all concentrations of TJA. However, *Fusarium oxysporum* was notably absent in various concentrations of Tomato Juice Agar (TJA) and Potato Dextrose Agar (PDA). In the case of PDA concentrations, *Aspergillus flavus, Aspergillus fumigatus,* and *Aspergillus niger* were uniformly isolated in all concentrations. Conversely, *Penicillium digitatum and Rhizopus stolonifer* were isolated in the 200 ml concentration.

Table 5. Occurrence of fungal isolate in different concentrations (TJA) and (PDA) for spoiled bread

Isolates	organisms	TJA concentrations/ml			PDA Concentrations/ml		
		200	300	400	200	300	400
1	*Aspergillus flavus*	+	-	-	+	+	+
2	*Aspergillus Fumigatus*	+	+	+	+	+	+
3	*Aspergillus Niger*	+	+	+	+	+	+
4	*Penicillium digitanum*	-	-	-	+	-	-
5	*Fusarium oxysporum*	-	-	-	-	-	-
6	*Rhizopus stolonifer*	+	+	+	+	-	-

DISCUSSION

The results obtained from isolating and characterizing fungal strains in different concentrations of Tomato Juice Agar (TJA) and Potato Dextrose Agar (PDA) mark a significant stride in developing mycological media. Based on the various concentrations (Table 1), the total mould count of TJA for refuse dumpsite soil ranged from 2-3cfu/ml, while for the spoiled bread ranged from 3-4cfu/ml. The variation in the range may be linked to the pH conditions of the (TJA) favoring the growth of particular molds over others. This agrees with the findings of Mbajiuka and Enya (2014), who isolated microorganisms associated with the deterioration of Tomato (Lycopersicon esculentum) and Pawpaw (Carica papaya) Fruits in Michael Okpara University of Agriculture Umudike, Abia State Nigeria and reported total fungi count at the range of 2-3cfu/ml.

The total mold count on potato dextrose agar (PDA) (Table 2) for the refuse dumpsite and spoiled bread. The results indicate that, across different concentrations of PDA, the mold count in refuse dumpsite soil ranges from 3-6 cfu/ml, while for spoiled bread, it ranges

from 3-5 cfu/ml. This result can be attributed to the nutrient abundance within PDA, providing an optimal environment for fungal proliferation. This is similar to the result of Kutawa *et al.* (2020), who reported total fungal at the range 3.8×10^5-$5.1 \times 10^1$for PDA used in the assessment of fungal species associated with tomato spoilage sold in Dutsin-ma Katsina state, Nigeria.

The isolates of fungi from refuse dumpsite soil and spoiled bread were *Aspergillus Flavus, Aspergillus Fumigatus,* Aspergillus *Niger, Penicillium digitatum, and F.xysporum spp* (Table 3). This study's findings align with the work of Aigbogun (2018), who also isolated *Aspergillus* spp using TJA and PDA. The findings of this study are also in conjunction with the work of Aliyu *et al.* (2017), who used tomato juice as a medium for growing fungi and isolated *Aspergillus* spp., *Penicillium spp., Fusarium* spp., and *Candida* spp,

The occurrence of mold isolates in different concentrations of TJA and PDA from refuse dumpsite soil (Table 4). *A. niger* and *R. stolonifer* were the only isolates found in the 3 different concentrations of both the TJA and PDA for the refuse dumpsite soil, while *F. oxysporum, P. digitatum* were isolated from various concentrations of PDA analyzed.

A. niger is a common environmental fungus. It is also relevant in agriculture. It decomposes organic matter and can have beneficial and harmful effects on crops. Its presence in refuse dumpsites can provide insights into the impact of human activities on microbial communities in these areas.

Rhizopus stolonifer is commonly known as black bread mold. It is a member of Zygomycota. It is one of the most common fungi globally and has a global distribution, although it is most commonly found in tropical and subtropical regions. It is a common agent of decomposition of stored foods (David *et al.*, 2015). Both media may have provided a variety of nutrient sources that support the growth of *A. niger* and *R. stolenifer*.

Fusarium spp. are ubiquitous and may be found in the soil, air, and plants. It is also known for causing vascular wilt diseases in many plant species. *Fusarium* spp. can cause mycotoxicosis in humans following ingesting food that the fungal organism has colonized. In humans, *Fusarium* spp. can also cause disease that is localized, focally invasive, or disseminated (Gupta *et al.*, 2000). It was isolated from all the various concentrations of the PDA. Its presence in a refuse dumpsite may indicate potential pathogenicity in diverse conditions. Potato Dextrose Agar contains a mixture of potato extract and dextrose, providing a rich and balanced nutrient source with carbohydrates, amino acids, vitamins, and minerals. *Fusarium oxysporum*, like other fungi, may have specific nutrient requirements. PDA may meet these requirements more effectively than TJA, promoting the growth of the fungus on PDA.

P. digitatum is a necrotrophic pathogen of citrus that can infect only through the wounds on the surfaces of fruits. The spores of *P. digitatum* can germinate and grow rapidly after contact with the wound tissue and complete their growth cycle within two days, resulting in citrus soft rot (Yang *et al.*, 2019). Penicillium species, including *P. digitatum*, are also known decomposers. Its presence in a refuse dumpsite could provide insights into its role in breaking down organic waste.

Aspergillus niger, *Rhizopus stolonifer*, and *Aspergillus flavus* were isolated from 200ml concentration of TJA. This could be because Tomato Juice Agar (TJA) contains nutrients derived from tomato juice, including sugars, vitamins, and minerals. Lower concentrations may provide a more optimal balance of nutrients for these fungi.

All the organisms isolated from the refuse dumpsite grow well in 200ml concentration of the PDA. This could be attributed to osmotic stress and toxic effects. Higher concentrations of agar in the medium can create osmotic stress for fungi. If the agar concentration is too high, it may limit the availability of water and nutrients to the fungi, affecting their growth. Also, extremely high agar concentrations might have toxic effects on the fungi. Agar is a polysaccharide, and excessively high concentrations may interfere with nutrient uptake and metabolism, hindering fungal growth.

The occurrence of mold isolates in different concentrations of TJA and PDA from spoiled bread (Table 5). *A. fumigatus* and *A. niger* were isolated from all the concentrations in both TJA and PDA from spoiled bread. *Aspergillus* species are known for their adaptability to various environmental conditions. They may thrive in various agar concentrations, demonstrating their ability to utilize different nutrient sources. Also, Agar concentrations can influence the water-holding capacity of the medium. Too high or too low concentrations may affect water availability to the fungi. The selected concentrations may provide an ideal water and nutrient uptake balance. The 200ml concentration of the TJA and PDA had a higher occurrence of organisms. Microorganisms require specific nutrients for growth and reproduction. The 200ml concentration of the tomato juice agar (TJA) and potato dextrose agar (PDA) may have had a higher nutrient concentration than the 300ml and 400ml concentrations, which might have enabled a more favorable environment for the organisms to thrive. Changes in the volume of the agar medium may influence osmotic pressure. Organisms may respond differently to variations in osmotic conditions, which could affect their growth. This is similar to the findings of Aliyu *et al.* (2017), who isolated *Aspergillus sp, Penicillium* sp, *Fusarium* sp, and *Candida* sp when Using Tomato Juice Supplemented With Glucose as a Medium for Growing Fungi and had *Aspergillus sp.* occurring higher.

The study successfully explored different concentrations of TJA and compared its performance with Potato Dextrose Agar (PDA) in cultivating fungi isolated from refuse dumpsite soil and spoiled bread. These findings also highlighted the significance of concentration variations in TJA, where certain fungi, such as *Aspergillus niger* and *Rhizopus stolonifer*, exhibited consistent growth across different concentrations. This suggests that the choice of concentration can influence the type of fungi cultivated, providing valuable insights for future experimentation and optimization.

Furthermore, the isolation of *Fusarium oxysporum* exclusively in PDA and the absence of certain fungi in TJA underscored the medium-specific preferences of some organisms. This emphasizes the importance of medium selection based on the targeted fungi and research objectives.

CONCLUSION

The study successfully formulated Tomato Juice Agar (TJA) as a mycological medium using fresh tomato juice, agar, glucose, and streptomycin. TJA effectively supported fungal growth, demonstrating its potential as an alternative to traditional media like Potato Dextrose Agar (PDA). TJA supported the growth of various fungi from refuse dumpsite soil and spoiled bread. *Aspergillus niger* and *Rhizopus stolonifer* were consistently isolated on TJA from both samples, while PDA supported a broader range of isolates, including *Penicillium digitatum* and *Fusarium oxysporum*. The total fungal counts on TJA ranged from 2-3 cfu/mL for refuse dumpsite soil and 3-4 cfu/mL for spoiled bread. PDA showed higher counts, ranging from 3-6 cfu/mL for refuse dumpsite soil and 3-5 cfu/mL for spoiled bread. TJA's effectiveness in supporting fungal growth highlights its potential for use in mycological studies, offering advantages such as cost-effectiveness and sustainability. While further research is needed to optimize TJA, this study lays the groundwork for developing alternative mycological media to advance microbiological research and diagnostics.

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