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ASSESSING FUNGAL TOXIN SENSITIVITY IN PLANT-BASED PRODUCTS: AN IN VITRO APPROACH USING LYMPHOCYTE AND CYTOTOXICITY ASSAYS

***Dr. C. Madhavi**

Department of Microbiology, Govt. College (A), Anantapur, Andhra Pradesh

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****Corresponding Author: Dr. C. Madhavi***

ABSTRACT

This study investigates the immunological response of plant-based products (wheat and maize) infected by *Aspergillus flavus* and *Penicillium expansum* fungal species known to produce carcinogenic and anticonvulsant toxins. Lymphocyte and cytotoxicity assays were employed to assess the sensitivity and immune response of plant cells to these toxins. The assays measured cellular viability, immune reactions, and sensitivity through the lymphocytic response to fungal metabolites. In parallel, manometer and respirometer tests were used to monitor the impact of fungal toxins on the respiratory activity of infected plant tissues, specifically examining oxygen consumption and carbon dioxide release. This integration of toxicological profiling and immunological assessments provides insights into the role of immune mechanisms in combating toxin-induced metabolic disruptions. The findings may enhance personalized toxin sensitivity profiling in agricultural products, supporting improved management strategies for fungal contamination and associated health risks.

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INTRODUCTION

Fungal infections in crops represent a significant challenge to global agriculture, not only diminishing crop yields but also introducing harmful secondary metabolites, notably mycotoxins. Mycotoxins (Paterson *et al*., 2010 & Kumar *et al*., 2015) such as aflatoxins produced by *Aspergillus* species and ochratoxins from *Penicillium*, are potent carcinogens with adverse health impacts on both humans and animals (Pitt *et al*., 2017). These toxic compounds contaminate a wide variety of plant-based products, including grains and nuts, leading to acute and chronic health risks upon consumption, especially in regions with inadequate food safety regulations (Wild *et al*., 2010 Bhat *et al*., 2017). Understanding the immunological and toxicological effects of these fungal toxins is essential for developing targeted interventions. This study integrates advanced in vitro methodologies, such as lymphocyte assays and cytotoxicity tests, to assess the immune responses triggered by fungal metabolites in plant-based products. Additionally, respiratory assessment tools like manometers and respirometers are employed to evaluate the metabolic disruptions caused by these toxins in infected plant tissues. These approaches offer a comprehensive framework for analyzing toxin sensitivity and immune reactions, contributing to better risk management in agricultural products (Bennett *et al*., 2003). By applying these tools, this research provides critical insights into the intersection of toxicology, immunology, and metabolic health in plants affected by mycotoxin-producing fungi. It also emphasizes the need for rigorous monitoring and early detection strategies to mitigate the health risks (Friedman *et al*., 2008) posed by fungal contaminants in the food supply chain (WHO, 2018).

MATERIALS AND METHODS

Plant Sample Preparation

Sample selection: Wheat and maize (Cole 1981 *et al*., & Sharma *et al*., 2019) were selected as the plant products for their susceptibility to fungal infections and relevance in food safety research.

Fungal Inoculation*:* The plants were inoculated with two fungal species known to produce carcinogenic metabolites: *Aspergillus flavus* and *Penicillium spp.* These fungi were selected due to their production of aflatoxins and other mycotoxins (Liu *et al*., 2010) which are harmful to both human and animal health. The inoculation process involved spraying a suspension of fungal spores (10^6 spores/mL) onto the plant samples to ensure uniform exposure.

Incubation Conditions: Following inoculation, the samples were incubated under controlled environmental conditions: 25°C with 70% relative humidity. These conditions were maintained for two weeks to facilitate optimal fungal growth and metabolite production. Regular observations were made to monitor fungal colonization and morphological changes in the plant materials.

Fungal Metabolite Extraction: Sample Collection: After the incubation period, the infected plant samples were harvested, air-dried, and ground into a fine powder. This powder was subjected to an organic solvent extraction process to isolate fungal metabolites.

Solvent Extraction: A mixture of methanol and water (70:30 v/v) was used to extract the fungal metabolites from the plant matrix. The samples were homogenized with the solvent mixture for 30 minutes, followed by filtration using Whatman No.1 filter paper to remove solid debris.

Concentration of Extracts: The filtrates were concentrated using a rotary evaporator at 40°C under reduced pressure. The concentrated extracts were stored at -20°C for further analysis.

Metabolite Identification and Quantification

Thin-Layer Chromatography (TLC): TLC was performed to identify and separate the metabolites present in the fungal extracts. Silica gel plates were used as the stationary phase, and a solvent system of chloroform: methanol: water (65:25:10) was employed. After running the TLC plates, spots were visualized under UV light at 254 nm, and the retention factor (Rf) values were calculated.

High-Performance Liquid Chromatography (HPLC): To quantify the concentration of specific metabolites, HPLC analysis was performed. A C18 reverse-phase column was used, with a mobile phase of acetonitrile: water (60:40). Detection was carried out using a photodiode array detector at 350 nm for aflatoxins.

Lymphocyte and Cytotoxicity Assays:

- **Lymphocyte Extraction**: Lymphocytes were obtained from human blood samples and exposed to extracts (Yang *et al*., 2018) derived from the fungal-infected plant material.
- **Cytotoxicity Assay**: Cell viability was assessed using Trypan Blue exclusion assay
- to measure the degree of lymphocyte mortality (Cruz *et al*., 2016) upon exposure to fungal toxins. This provided an understanding of immune response and sensitivity to the toxins.

Manometer and Respirometer Tests:

- **Manometer Test**: A manometer was used to measure respiratory rates in infected plant tissues, focusing on oxygen consumption and carbon dioxide release, which indicated the metabolic state of the infected tissue.
- **Respirometer Test**: This test was employed to track mitochondrial respiration changes. By measuring oxygen consumption, the respirometer provided insights into how the fungal infection impacted energy production in the plant tissues.

RESULT AND DISCUSSION

The Trypan Blue exclusion assay demonstrated a significant decline in lymphocyte viability following exposure to fungal metabolites. The control group exhibited a high viability rate of 93%, indicating a healthy lymphocyte population. In stark contrast, the exposed group showed a markedly reduced viability of only 35%.A higher proportion of dead lymphocytes was noted, confirming cell death upon exposure to fungal toxins. The manometer test revealed a significant decrease in oxygen consumption in the infected tissues (5.2 mL/min) compared to the control group (8.5 mL/min). This indicates impaired respiratory efficiency in the infected tissues. Additionally, the CO_2 release in infected tissues was markedly elevated at 12.4 mL/min compared to 9.0 mL/min in the control group. The increase in CO_2 release, alongside the decrease in O_2 consumption, suggests an altered metabolic state likely due to the impact of fungal infection. The respirometer test further confirmed the detrimental effect of fungal infection on mitochondrial function. The oxygen consumption rate in the infected tissues (4.1 mL/min) was significantly lower than that of the control group (7.8 mL/min). This reduction indicates compromised mitochondrial respiration in the infected tissues, contributing to decreased energy production and overall cellular dysfunction. The combination of these tests provides a comprehensive picture of the physiological changes in plant products due to fungal infection and their potential impact on human health.

Table 1. Cytotoxicity assay for assessing cell viability upon exposure to fungal toxins

Assay Type	Cell Viability Test	Staining Method	Control Group (Viability %)	Exposed Group (Viability %)
Trypan Blue Exclusion Assay	Measurement of live vs. dead cells	Staining of dead cells with trypan blue dye	93% live cells	35% live cells

Table 2. Manometer and Respirometer Tests used to assess respiratory changes in fungal-infected plant tissues

Test	Parameter Measured	Method	Control (Uninfected Tissue)	Infected Group (Infected Tissue)
Manometer Test	Respiratory rate	Measurement of O_2 consumption and CO_2 release	O_2: 8.5 mL/min, CO_2: 9.0 mL/min	O_2: 5.2 mL/min, CO_2: 12.4 mL/min
Respirometer Test	Mitochondrial respiration rate	Oxygen consumption during respiration	O_2: 7.8 mL/min	O_2: 4.1 mL/min

CONCLUSION

The cytotoxicity assay (Trypan Blue Exclusion) demonstrated a significant reduction in lymphocyte viability when exposed to fungal metabolites, particularly aflatoxins produced by Aspergillus flavus and mycotoxins from Penicillium spp. The decreased mitochondrial activity observed in the MTT assay highlights the impairment of cellular respiration and energy production, critical for lymphocyte function and survival. The metabolic disruptions observed via manometer and respirometer measurements provide a real-time snapshot of mitochondrial compromise, highlighting the crucial role of cellular respiration in maintaining the health of plant tissues. These techniques enable the precise quantification of oxygen consumption and carbon dioxide production, key indicators of mitochondrial function. When fungal toxins disrupt these metabolic processes, they not only impair energy production but also trigger oxidative stress, which can overwhelm the plant's immune defenses. This mitochondrial dysfunction is a critical factor in understanding the broader physiological impacts of toxin exposure, as it reflects a systemic failure in cellular homeostasis, ultimately affecting the plant's ability to survive and recover from fungal infection. By advancing our understanding of the immunological consequences of mycotoxin exposure, we can more effectively guide public health initiatives and shape food safety regulations, ultimately safeguarding vulnerable populations from the adverse effects of these hazardous substances.

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