Review Paper: Survey and Detection of Seed-Borne Microflora of *Pisum Sativum*

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ABSTRACT

Pea (*Pisum sativum L.)*, a globally important legume, frequently suffers from reduced productivity due to various seed-borne microorganisms, including fungi, bacteria, and viruses. These pathogens can cause significant damage, leading to poor germination, seedling diseases, root rot, and lower yields. This extensive review compiles the latest information on the common microbes found in pea seeds and critically examines both traditional and modern methods for detecting these organisms. Recent research highlights major pathogens such as Fusarium, Alternaria, Ascochyta, Rhizoctonia solani fungi, as well as the bacterium Pseudomonas syringae pv. pisi and the economically damaging Pea Seed-borne Mosaic Virus (PSbMV). The review stresses that ongoing improvements in detection techniques are crucial for protecting seed health, preventing widespread disease, and maintaining sustainable pea production amidst evolving farming methods and new biological threats.

INTRODUCTION

Pea (*Pisum sativum L.)* is a vital crop globally, valued for its high protein content, role as animal feed, and nitrogen fixation in sustainable agriculture. However, despite its importance, pea farming faces ongoing challenges from diseases, often originating from pathogens carried in the seeds (Agarwal & Sinclair, 1997). These microorganisms, either present on the seed surface or within its tissues, are the main cause of disease spread over long distances, resulting in substantial losses in later harvests (Patra, 2023).

Seed-borne microbes can severely impact pea crops, causing poor germination, weak seedlings, and severe infections in mature plants. This ultimately results in significant yield loss and a decline in seed quality (Gaurilčikienė et al., 2012; Patra, 2023). Therefore, a thorough understanding of common seed-borne microorganisms and the development of effective detection techniques are essential for managing diseases and ensuring the long-term viability of pea cultivation.

This review aims to:

• Provide an updated overview of the most common and economically significant fungi, bacteria, and viruses found in pea seeds.

• Critically examine the conventional, serological, and molecular methods for detecting these pathogens, highlighting their latest improvements, benefits, and limitations.

• Emphasize the vital role of seed health testing in disease management strategies for pea crops.

Pea Seed Microorganisms: An Overview

Pea seeds host a diverse range of microorganisms, including beneficial microbes, parasitic organisms, and strict pathogens. Each of these affects seed quality and plant health differently.

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Fungal Pathogens

Fungi are the most significant group of pea seed pathogens, often causing damping-off, root rots, blights, and wilts.

• **Fusarium species**: Fusarium oxysporum and F. solani are major concerns, leading to Fusarium wilt and root rot. Recent studies also highlight F. proliferatum and F. equiseti in symptomatic pea seeds (Mirković et al., 2023).

• **Ascochyta complex:** This includes Ascochyta pisi, Phoma pinodella, and Mycosphaerella pinodes, responsible for Ascochyta blight. Infected seeds may show shriveling and discoloration, though symptomless carriers complicate detection (Gaurilčikienė et al., 2012).

• **Alternaria species:** Alternaria alternata can reduce seed germination and vigor, and cause leaf spots and blight (Kesharwani et al., 2018).

• **Rhizoctonia solani:** Though primarily soil-borne, R. solani can be carried on seeds, leading to damping-off and root rot (Uysal et al., 2011).

• **Aspergillus and Penicillium species**: These storage fungi cause seed decay and reduced viability under humid conditions (Kesharwani et al., 2018).

• **Pythium species**: While mostly soil-borne, some Pythium species can be seed-surface contaminants causing damping-off (CABI Compendium, n.d.).

• **Botrytis cinerea (Grey Mould):** This widespread fungus can infect pea seeds, causing decay and seedling blight under favorable conditions (ResearchGate, 2015).

Bacterial Pathogens

Bacterial pathogens, while fewer in variety than fungi, can cause severe diseases that are difficult to eliminate from seeds.

• **Pseudomonas syringae pv. pisi (Bacterial Blight):** The most critical bacterial seed-borne pathogen of pea, causing blight and lesions on plant parts. Its ability to survive on or in seeds makes it a major source of infection (APS, n.d.; Biblioteka Nauki, 2012).

• **Erwinia rhapontici (Pink Seed):** Causes a distinct pink discoloration in seeds, affecting seedling emergence (APS, n.d.).

• **Human Pathogens:** Concern over human pathogens like Escherichia coli (e.g., O104:H4) in seeds, especially for sprouting, is rising (Kennedale et al., 2020).

Viral Pathogens

Seed-borne viruses cause systemic infections that are challenging to control.

• **Pea Seed-borne Mosaic Virus (PSbMV):** The most economically damaging seed-borne virus for peas, causing mosaic patterns, stunted growth, and malformed pods. Detection and breeding for resistance are crucial (APS Journals, 2022; ResearchGate, 2015).

• **Bean Yellow Mosaic Virus (BYMV) and Pea Enation Mosaic Virus (PEMV):** These viruses can also infect peas, contributing to yield losses (ResearchGate, 2015).

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Advances in Detection Methods

Detection methods for seed health testing have significantly advanced, from simple visual checks to cutting-edge molecular techniques. These methods vary in their strengths, limitations, and applications. A comparative table summarizing these detection methods is provided below.

Method	Strengths	Limitations	Ideal Use Case
Visual Inspection	Simple, low-cost	Low sensitivity, can't detect internal infections	Preliminary screening for obvious symptoms
Incubation Methods	Cost-effective, reliable	May miss low-level infections	Routine screening of fungal pathogens
Serological Methods	High specificity, fast	Requires specific antibodies	Virus and certain bacterial pathogen detection
Molecular Methods (PCR)	High sensitivity, accurate	Requires technical expertise	Detection of low-level or hard-to-culture pathogens

Traditional Methods

Traditional seed health testing methods remain cost-effective for routine screening.

• **Visual Inspection:** Effective for spotting obvious symptoms, such as discoloration or fungal structures.

• **Incubation Methods: T**he standard blotter method and agar plate method are widely used for fungal detection.

• **Washing Test:** Useful for detecting surface contaminants like fungal spores or bacteria.

•**Grow-on Test:** Confirms the pathogenicity of detected microorganisms by observing disease symptoms in seedlings.

Serological Methods

Serological methods provide higher specificity than traditional techniques, offering faster results.

• **Enzyme-Linked Immunosorbent Assay (ELISA):** Sensitive and quantitative, used for detecting viruses and some bacterial pathogens.

• **Immunofluorescence Microscopy:** Effective for detecting bacterial cells using fluorescently tagged antibodies.

Molecular Methods

Molecular techniques are the gold standard for pathogen detection, offering unmatched sensitivity and specificity.

• **Polymerase Chain Reaction (PCR):** Used for detecting specific pathogens, including P. syringae and PSbMV.

• **Real-time PCR (qPCR):** Quantifies pathogen DNA, providing detailed information on infection levels.

• **Multiplex PCR:** Simultaneously detects multiple pathogens, saving time and resources.

• **High-Throughput Sequencing (HTS):** Provides a comprehensive view of the seed microbiome, detecting all microbial species present, including emerging pathogens.

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Why Seed Health Testing is Crucial for Pea Production

Seed health testing is a vital part of Integrated Disease Management (IDM) for pea production.

• **Stopping Disease Spread:** Early detection prevents pathogen introduction to new areas.

• **Ensuring Seed Quality:** Healthy seeds are essential for optimal germination and plant growth.

• **Meeting Trade Standards:** Seed health standards ensure compliance with phytosanitary regulations.

• **Supporting Breeding Programs:** Knowledge of seed-borne pathogens is crucial for developing resistant pea varieties.

• **Reducing Chemical Use:** By ensuring healthy seeds, the need for chemical seed treatments can be minimized.

Future Trends and Directions

The field of seed pathology is evolving rapidly.

• **New Pathogens and Microbial Shifts:** Climate change and changing farming practices may lead to shifts in pathogen prevalence.

• **Portable Diagnostics:** User-friendly, portable diagnostic kits will enable on-site testing for rapid decision-making.

• **Bioinformatics and AI:** Data analysis from advanced sequencing methods will be enhanced by AI algorithms to improve pathogen detection.

• **Biological Control and Seed Priming:** Bio-priming using beneficial microorganisms offers an eco-friendly alternative to chemical treatments.

CONCLUSION

Healthy pea seeds are crucial for successful pea production, but seed-borne microorganisms pose a continuous threat. This review highlights the key fungal, bacterial, and viral pathogens affecting peas and discusses a range of detection methods. The future of seed health testing is likely to be driven by advancements in molecular diagnostics, portable testing, and the integration of AI and bioinformatics. Effective seed health testing is essential for protecting pea yields, ensuring food security, and supporting sustainable agricultural practices.

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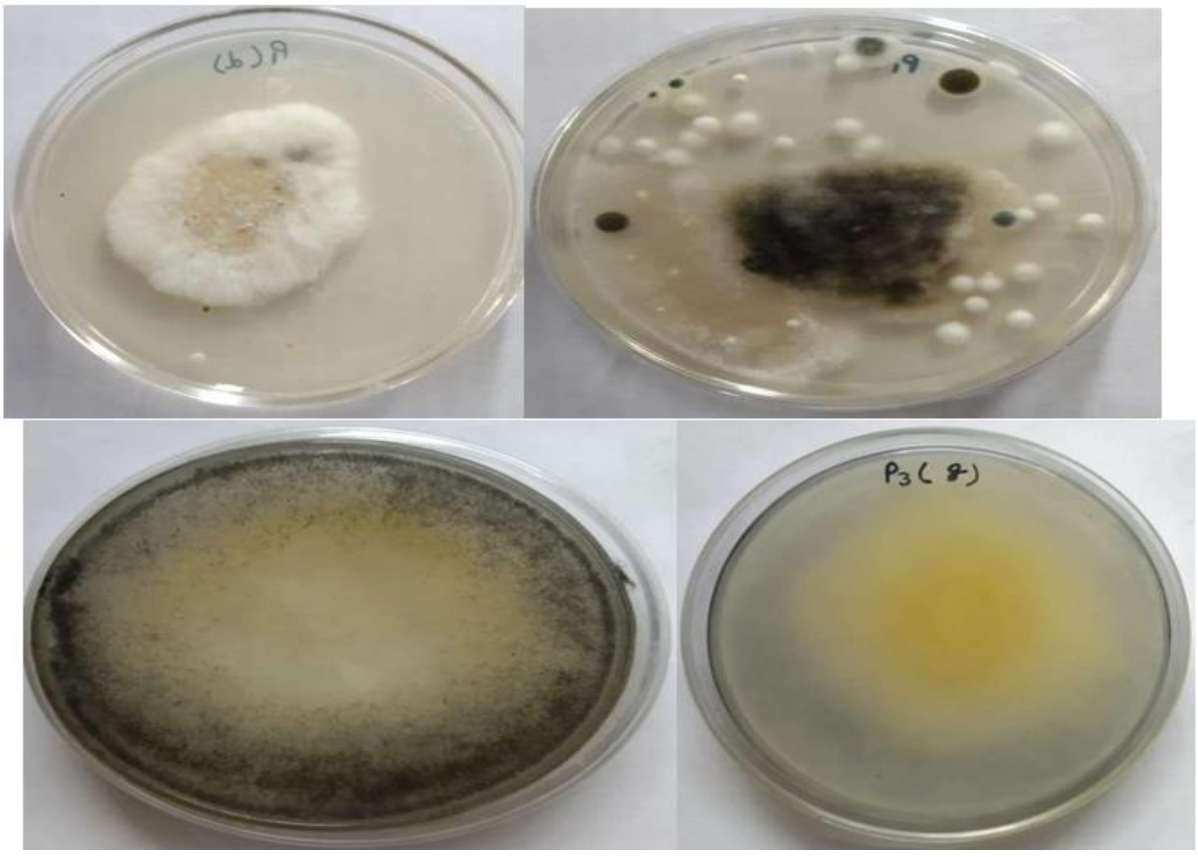

Figure 1: Isolated pure fungus culture

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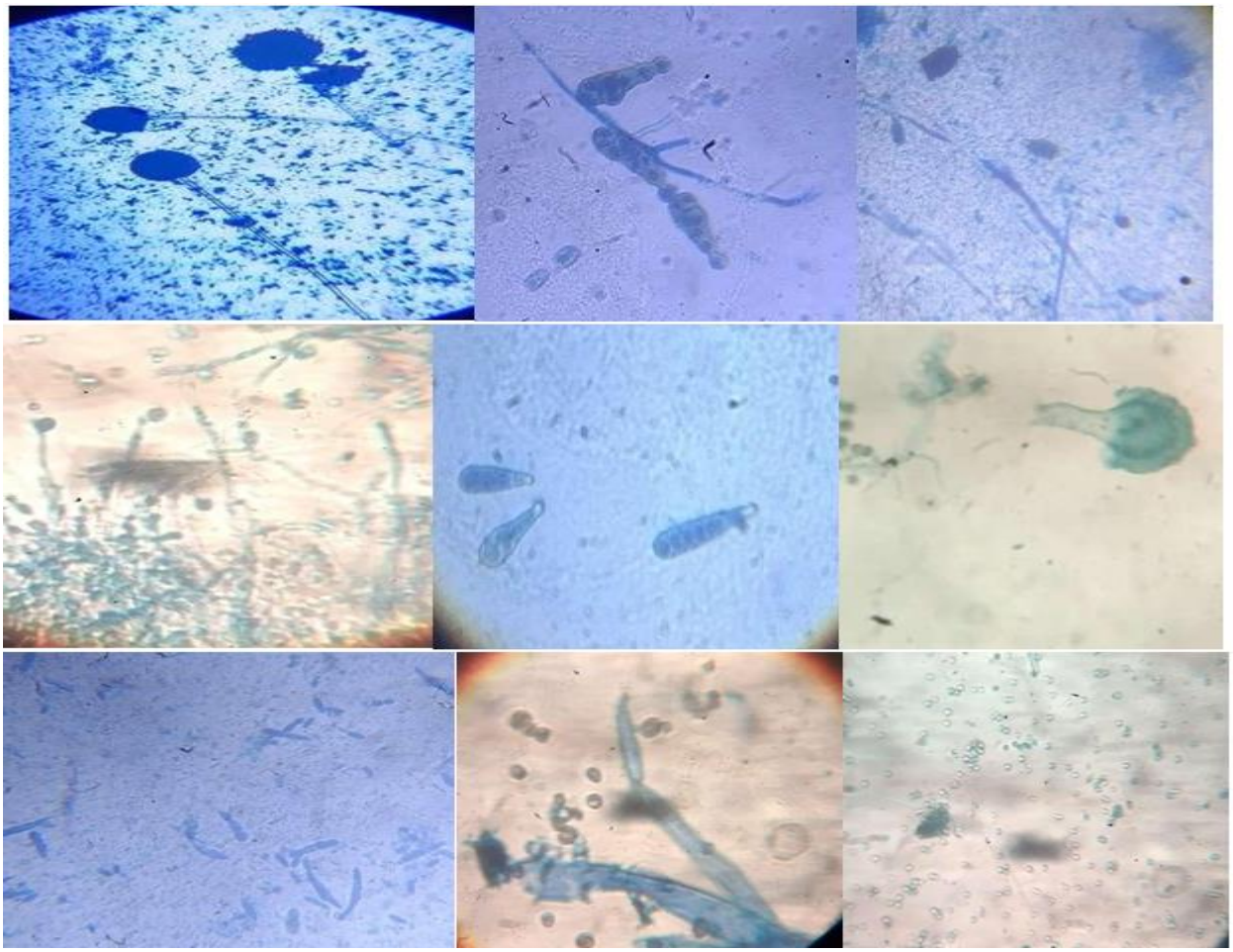

Figure 2: Isolated fungus microscopy

Fungus identification: During microscopy observation 9 fungi

Some in showing (*A. niger*, *Penicillium* spp., *F. moniliforme*, *Alternaria* spp., *Phythium* spp.) were identified.