

SOURCE OF INOCULUM FOR SOME SELECTED FUNGAL DISEASES OF PISUM SATIVUM LINN. IN ZARIA, KADUNA STATE NIGERIA

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ABSTRACT

Field pea (*Pisum sativum*) is an important crop for food, feed, and rotation systems. This study investigated sources of inoculum for eight fungal diseases—rust, powdery mildew, wilt, pod rot, root rot, leaf spot, leaf blight, and gray mold—in irrigated fields of Zaria, Kaduna State, Nigeria. Seeds, soil, and hay were collected postharvest from the villages of Katanga and Shika Dam. Fungal isolation was performed using washing and direct plating techniques on Potato Dextrose Agar, with identification based on macroscopic and microscopic features. Results revealed that rust and pod rot inocula originated from seeds, soil, and debris, whereas powdery mildew, leaf spot, and gray mold persisted only on plant debris. Rust (*Uromyces* spp.) showed the highest occurrence (80%), followed by wilt (*Fusarium* spp., 70%), leaf blight and root rot (*Rhizoctonia* sp., 50%), powdery mildew (*Erysiphe* spp., 40%), and leaf spot (*Alternaria* sp., 30%). Pod rot (*Thielavia*), gray mold (*Botrytis*), and *Pseudallescheria* spp. (wilt) were least frequent (10%). These findings demonstrate that soil, seeds, and debris serve as major reservoirs of inoculum for *P. sativum* fungal diseases. Effective field sanitation and seed treatment are recommended to minimize pathogen transmission and safeguard subsequent plantings.

Keywords: *Pisum sativum*, PDA, Inoculum

INTRODUCTION

Field pea is primarily used for human consumption or as livestock feed. It is a grain legume commonly used throughout the world in human cereal-grain diets; it has high levels of the amino acids lysine and tryptophan, which are relatively low in cereal grains. Field pea contains approximately 21 to 25 % protein, high levels of carbohydrates and low fiber. It contains 86–87% total digestible nutrients, making it an excellent livestock feed. It contains 5- 20% less trypsin inhibitor than soybean (McKay, 2003). This allows it to be fed directly to livestock without going through the extrusion heating process. Field pea is often cracked or ground and added to cereal-grain rotations (Chen *et al.*, 2006). The crop is grown mainly as a cash crop in Nigeria; for example, in 2017, a bag was sold at ₦ 250,000.00 (\$694.44) per 100 kg, and to this day the price continues to appreciate (Ibrahim *et al.*, 2020). The plant was found to be cultivated under irrigation in a few states in the Northern Guinea Savanna of Nigeria, especially Kaduna, Katsina and Plateau States (Ibrahim *et al.*, 2022).

Fungi have been shown to induce many diseases in field pea wherever the crop is grown (Laflamme, 1998). Members of the Dikaryomycota primarily cause fungal diseases. They have caused

famines and ruined the economies of large areas, including entire countries, causing yield losses of up to 100% depending on the stage of plant growth (Upadhyay & Singh, 1994; Chand *et al.*, 2004; Agrios, 2005; Voegele, 2006; Shivani, 2017).

The source of inoculum for fungal diseases can be soil, seed or debris, depending on the fungal species. Determining the source of inoculum for fungal organisms is a prerequisite to the management of fungal diseases. Therefore, this study aims to determine the source of inoculum for selected fungal diseases of *P. sativum* in Zaria, Kaduna State, Nigeria.

MATERIALS AND METHODS

Sample Collection

Seeds, soil and hay of *P. sativum* were collected from irrigated fields in Zaria (Katanga and Shika Dam villages) after the April harvest. Katanga village lies at Latitude 11°10'13"N and Longitude 7°46'39"E, while Shika Dam village lies at Latitude 11°08'24"N and Longitude 7°45'54"E. The collected seeds were taken to the Herbarium Department of Botany at Ahmadu Bello University for identification.

Preparation of media

Commercially formulated Potato Dextrose Agar (PDA) was prepared as described by the manufacturer's instructions (Oxoid, England) and used throughout the study. 40 g of Potato Dextrose Agar was weighed, dissolved in 1 liter of distilled water, and melted in a water bath. The medium was sterilized in an autoclave at 121°C for 15 minutes.

Isolation of fungi from seed/ field soil/ hay(plant debris)

Washing technique: Two grams of seeds were weighed and put in a test tube containing 10 ML of sterile distilled water. It was shaken for 10 minutes on a mechanical shaker. Using a sterile micropipette, one drop of the suspension was placed on a glass slide, covered with a coverslip and observed under the microscope at x 40 magnification for the presence of fungal spores and conidia, as described by Shivani (2017).

Fungi were isolated through direct soil plating. Soil samples (0.05 g) were suspended in sterile water and dispersed across the bottom of sterile Petri dishes. Sterile, melted, cooled PDA agar was added, then rotated to distribute the particles throughout the medium and incubated at room temperature for 5 to 7 days. It was observed daily for the presence of fungal colonies (Marley, 2013). Direct tissue plating was used for hay/plant debris (Mungo, 1996). Infected hay samples were cut with sterile surgical blades. The pieces were surface-sterilized in 0.1% mercuric chloride (HgCl) for

3 minutes and rinsed 3 times with sterile distilled water. The hay was then plated on Petri dishes (four lesions per plate) containing Potato Dextrose Agar (PDA) amended with streptomycin sulphate (1.5 g/l) to inhibit bacterial growth. Each sample was in duplicate. The Petri dishes were incubated at room temperature. Observations were made daily for seven days for the presence of colonies of fungal growth. The fungal colonies were subcultured on fresh sterile PDA for subsequent identification.

Identification of Fungi

Spores were collected from single rust pustules directly from the *P. sativum* plant. The collected spores were mounted in lactophenol cotton blue and observed under the microscope. The color, shape and surface morphology of the spores were determined. 100 spores of each spore type were measured at x40 magnification (Emeran *et al.*, 2005). They were identified using keys developed by Shivas *et al.* (2014).

For powdery mildew diseases, temporary slides were prepared in the field using a piece of clear tape (smaller than the slide). It was held on one end of the tape and lightly smoothed the rest of the tape, sticky side down, over the infected plant part. The tape was placed sticky side down on a drop of water/ lactophenol cotton blue on the microscopic slide. The slide was observed under the microscope at x 40 magnification. Dried herbarium samples were put into a drop of lactic acid and gently heated before examination. The color, shape and surface morphology of the conidia were determined. The length and width of 100 conidia and conidiophores were measured in micrometers at x 40 magnification. Identification was performed using keys developed by Haffer *et al.* (2006) and Braun *et al.* (2017).

Identification of other fungal diseases was carried out using both macroscopic and microscopic observations of pure cultures. Fungi were identified by macroscopic characters such as colony texture and color, as well as cultural characteristics on PDA. Fungi were identified by examining microscopic characters, including conidial type and conidiophore morphology. Confirmation of the fungal identification was done using identification keys (Barnett & Hunter, 1999) and by comparison with already described isolates in the Department of Crop Protection diagnostic unit, Institute of Agricultural Research, ABU Zaria.

The percentage occurrence of fungi isolated was determined using the formula below (Ibrahim *et al.*, 2025a).

$$\text{Percentage occurrence} = \frac{\text{Frequency of occurrence of fungi}}{\text{Total number of fungi}} \times 100$$

RESULTS

Both obligate and facultative fungi causing diseases on field pea were encountered. The obligate fungi were identified through direct scraping; they include (Rust and Powdery mildew diseases. From rust pustules, urediospores and teliospores were observed, which are single-celled, ellipsoidal and obovoidal. Urediospores are yellowish-brown in color with 2- 4 equatorial or subequatorial germ pores, finely echinulate, with a length of 15– 24.5 µm and a width of 14.4– 20.1 µm; the teliospores are dark brown, smooth, with a thick apex. They measure 21.6–28.8 µm in length and 19.2–24 µm in width; the size and type confirmed those of *Uromyces* sp. From powdery mildew diseases, the size of conidia of *Oidium* sp. (*Erysiphe* sp) ranged from 28 x 12 µm to 41 x 19 µm in length/width ratios. The length of the conidiophore ranged from 70 to 84 µm, and the width from 6 to 8.4 µm, as shown in Table 1 and Plate 1. The facultative fungi: Whitish mycelia with pink at the base reverse side of the plate showed brown color, when observed under the microscope macro and micro conidia were present, macro conidia is curved and of average length with size range of 31.5 – 59.4 µm in length and width of 4.6 – 6.2 µm, micro conidia is 1- celled with size range of 7.2 – 15 µm length and width of 2.4 -3.9 µm, the organism was identified as *Fusarium* sp. *Pseudallecheria* sp. exhibited cultural characters of milk-raised, cottony mycelia on both the upper and reverse sides of PDA-containing plates; conidia were present. Some of the conidia were curved, measuring 8–40 x 5–10 µm, with length/width ratios as shown in Table 1 and Plates 2 and 3. *Rhizoctonia solani* had cultural characters of black mycelia covering PDA on both the upper and reverse sides of the plate, and microscopic characters of light brown mycelia, long and septate, with branches usually arising from the main hyphae. *Alternaria alternata* had dark brown, cottony growth with black at the base of PDA; microscopic characters: the conidia are dark, with both cross and longitudinal septa, and are variously shaped. *Thielavia* sp. on PDA had whitish, cottony mycelia that were light brown at the base; conidia are small, 1-celled, hyaline, ovoid to elongate; pycnidia are round and black, as shown in Table 1, Plates 2 and 3. *Botrytis* sp. had gray cottony growth on both the upper and reverse sides of the plate; conidiophores were tall, slender, hyaline and branched irregularly in the upper portion, bearing clusters of conidia, as shown in Table 1 and Plates 2 and 3.

Table 1: Identification of fungal isolates from soil, seed and debris of *P. sativum*

Macroscopic characters	Microscopic Characters	Probable Fungi
	Urediospores and teliospores are single-celled, ellipsoidal. Teliospores are dark brown; urediospores are yellowish. Urediospore: Length = 15– 24.5 µm. Width = 14.4– 20.1 µm. Teliospore: length 21.6 – 28.8 µm, width 19.2- 24 µm	<i>Uromyces</i> spp
	Conidia ellipsoidal or ovoid, hyaline, produced singly on aseptate conidiophores that arise from epiphytic hyphae. Length ranges from 28 µm to 41µm. Width ranges from 12µm to 19µm.	<i>Erysiphe</i> sp.
Whitish mycelia with a pink base. The reverse side of the plate shows brown growth.	Both macro- and microconidia. The macroconidia are curved and of average length; size ranges from 31.5–59 µm in length, with widths of 4–6 µm. Microconidia 1-celled, with a size range of 7.2 – 15 µm in length and 2.4- 3.9 µm in width.	<i>Fusarium</i> sp.

Milk-raised cottony mycelia on both the upper and lower surfaces. Reverse side milk-colored	Conidia present. Some of the conidia are curved; size ranges from 8–40 × 5–10 µm, with length/width ratios.	<i>Pseudallescheria</i> sp.
Black mycelia covering both the upper and reverse sides of the plate.	Cells of mycelium: long, septate branches. 5-9 µm wide	<i>Rhizoctonia</i> sp.
Dark brown cottony growth with black at the base. Reverse side black in color.	Conidia dark with both cross and longitudinal septa, 18- 45 × 2.3- 3.5 µm; length-to-width ratio.	<i>Alternaria</i> sp.
Whitish cottony mycelia that show light brown on the reverse side.	Ascospores, 1-celled, hyaline, ovoid, 9-15 x 6- 8 µm; length-to-width ratio. Cleistothecia black, 75- 170 µm in diameter	<i>Thielavia</i> sp.
Grey cottony growth on both upper and lower surfaces. The reverse side of the plate shows a milky color.	Conidiophore: tall, slender, hyaline, branched. Conidia: hyaline, 1-celled, ovoid.	<i>Botrytis</i> sp.

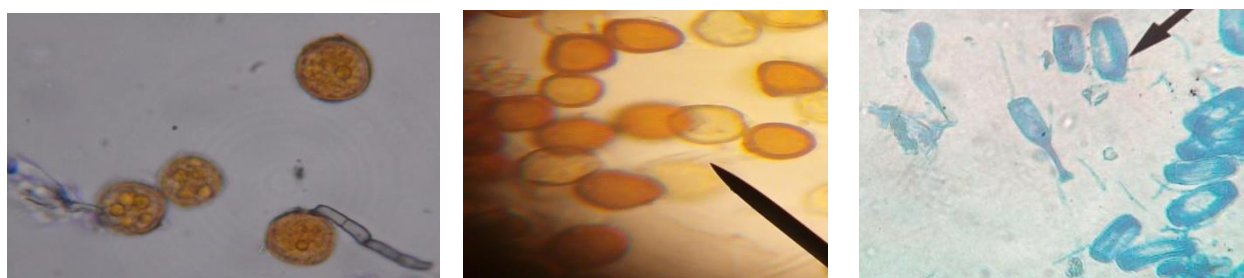


Plate 1: A= Urediospores of *Uromyces* sp. B= Teliospores of *Uromyces* sp. C= Conidiophore bearing conidia of *Erysiphe* sp. Stain = Lactophenol cotton blue, Mg x40.

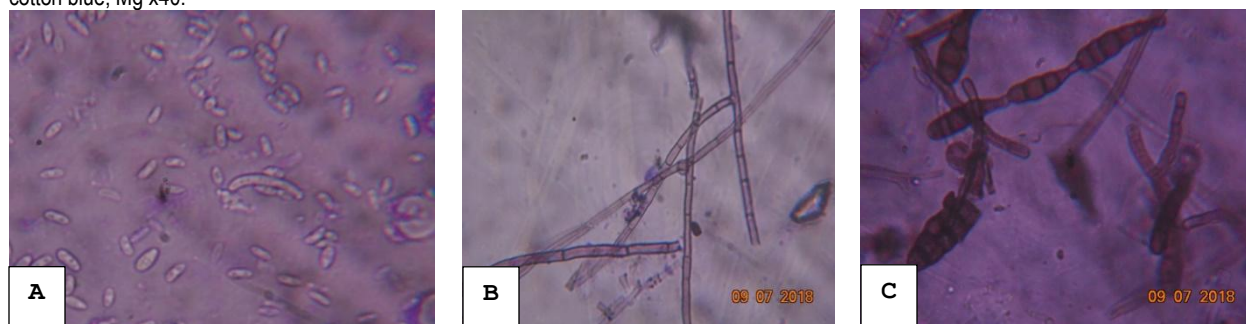


Plate 2: A= *Fusarium* sp. (microconidia and macroconidia) B = *Rhizoctonia* sp. (Branched septate hyphae) C= *Alternaria* sp. (Conidia with both cross and longitudinal septa) Stain = Lactophenol cotton blue, Mg x40.

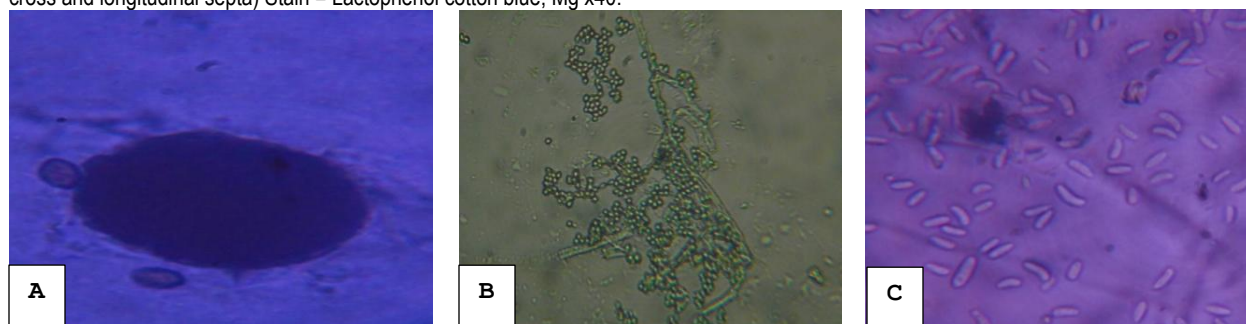


Plate 3: A= *Thielavia* sp. (Cleistothecia black, Ascospore one cell) B= *Botrytis* sp. (Conidia hyaline one cell), C = *Pseudallescheria* sp. (Conidia)

Isolation of fungi from seed, soil and hay/debris showed the presence of teliospores and urediniospores of rust disease on the seeds, soil and hay/debris. At the same time, conidia of powdery mildew were only found on hay/debris. *Rhizoctonia* and *Fusarium*

spp. were isolated from the soil as well as plant debris. *Alternaria* and *Botrytis* spp. were isolated from plant debris. *Thielavia* sp. was isolated from seeds, soil and debris; *Pseudallescheria* was only isolated from the soil, as shown in Table 2.

Table 2: Source of Inoculum for the Fungal Diseases of *P. sativum*

s/no	Disease	Fungi	SOURCE OF INOCULUM		
			Seed	Soil	Hay/Debris
1.	Rust	<i>Uromyces</i> spp.	+	+	+
2.	Powdery mildew	<i>Erysiphe</i> spp.	-	-	+
3.	Wilt	<i>Fusarium</i> spp.	-	+	+
		<i>Pseudallescheria</i> sp.	-	+	-
4.	Leaf Blight	<i>Rhizoctonia</i> sp.	-	+	+
5.	Leaf spot	<i>Alternaria</i> sp.	-	-	+
6.	Pod rot	<i>Thielavia</i> sp.	+	+	+
7.	Root rot	<i>Rhizoctonia</i> sp.	-	+	+
8.	Gray mold	<i>Botrytis</i> sp.	-	-	+

Key: + = Presence; - = Absent

Rust (*Uromyces* spp.) was found to have a high percentage occurrence (80 %), wilt (*Fusarium* spp: 70%), leaf blight and root rot (*Rhizoctonia solani*: 50 %), followed by powdery mildew (*Erysiphe* spp: 40 %), leaf spot (*Alternaria* sp.: 30%), while pod rot (*Thielavia*), *Botrytis* (gray mold) and *Pseudallescheria* spp. (wilt) had the least (10%), as shown in Fig. 1

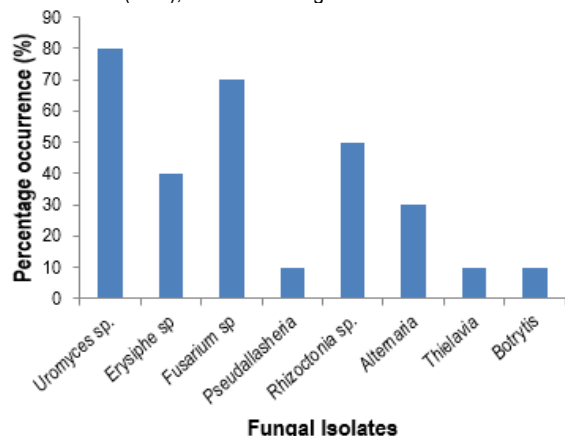


Fig 1: Percentage Occurrence of Fungal isolates

DISCUSSION

The fungal spores, urediospores and teliospores, have been identified from the seeds in these studies, which is in line with the report of Emeran (2005) and Mycobank (2019), who reported rust diseases to be seed-borne. The morphology of the urediniospores and teliospores of the observed rust was similar to that reported by Gasich (2004). *Uromyces* sp. is a heteroecious fungus (Mycobank, 2019). A high incidence of rust, wilt, and powdery mildew diseases was recorded on pea plant sources; this report is in line with the reports of Nongmaithem *et al.*, 2017; Upendra *et al.*, 2017; Ibrahim *et al.*, 2020a; Ibrahim *et al.*, 2020b and Ibrahim *et al.*, 2025b on vegetable pea, field pea and broad bean.

Fusarium sp., a pathogen of wilt diseases, was identified in this study, which is consistent with the following reports: the fungal pathogen that causes wilt diseases is soil-borne, and chlamydospores remain infective in the soil for 10 years (Cousin, 1997). Meena *et al.* (2011) reported a high incidence of wilt in India. Also, Ibrahim *et al.* (2022) reported a high incidence of both *F. solani* and *F. oxysporum* on field pea in the northern Guinea Savanna of Nigeria.

The identification of *Pseudallescheria*, *Alternaria*, *Thielavia*, *Rhizoctonia* and *Botrytis* spp., both by morphology on Petri dishes and by microscopic characters such as conidial size and shape and from different sources of inoculum and plants, is in line with the report of Babu *et al.* (2014). Watanabe (2002); Abdulaziz and Rashad (2010); Mathew *et al.* (2012); Ibrahim *et al.* (2022).

Finding these fungal inoculum on seeds, soil and debris in this study may suggest possible transmission of the fungal pathogens to field pea plants as the plants are intercropped or crop rotation with other susceptible plants on same fields in these locations as other researchers have reported the pathogens on other plants species (Laxmirawat *et al.*, 2014); Sohair *et al.*, 2015); Irena *et al.*, 2016; Mahesh *et al.*, 2016; *Botrytis* blight, 2018; Donald, 2019; Ibrahim *et al.*, 2025a). Since field pea seeds are being imported into Nigeria, they might have carried fungal spores, as urediniospores and teliospores have been identified on these seeds in these studies.

Conclusion

The sources of inoculum for rust and pod rot diseases were found to be soil, seed and debris, while soil and debris were the sources of inoculum for wilt and root rot. Debris was found to be the source of inoculum for leaf spot, powdery mildew and gray mold diseases.

Recommendation

Seeds should be properly treated before planting. Quarantine measures should be implemented for field pea seeds entering the country to prevent the spread of fungal pathogens.

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