Occurrence of pea root rot in different regions of Himachal Pradesh and identification of pathogen associated with disease

Abstract

Pea root rothas emerged as a significantconcern in select cultivation regions of Himachal Pradesh, with varying levels of disease incidence recorded across districts. In this study, disease incidence was quantified, with notable rates documented in Bir, Chougan, and Kukumseri. The widespread presence of pea root rot, underscores the severity of the issue. Previous research has identified *Fusarium solani* f. sp. *pisi* as a primary pathogen. The morphological, cultural, and pathogenic evaluations, confirm the involvement of *Fusarium solani* f. sp. *pisi* in pea root rot. Economic implications of this disease complex are significant, necessitating comprehensive understanding and management strategies. The methodological approach, including pathogenicity tests using Hoagland's solution, offers insights into disease progression and symptomatology. These findings contribute valuable insights into the biology and management of pea root rot, essential for developing effective disease control strategies in pea cultivation..

Keywords: Root rot, Incidence, *Fusarium solani*

1 INTRODUCTION:

Pea (*Pisum sativum* L.)
stands as an essential crop globally, cherished for its nutritional value and adaptability to various climatic conditions. Its cultivation spans diverse regions, with particular prominence in cooler climates, where it serves as a staple vegetable and a significant source of protein-rich pulses. In regions such as Lahaul and Spiti, Kinnaur, Shimla, Kullu, Mandi, and Kangra in Himachal Pradesh, peas occupy a pivotal position in agricultural practices, contributing substantially to the local economy and dietary diversity (Bhardwaj and Vikram, 2004). The cultivation of peas not only provides sustenance but also represents an economic lifeline for many farming communities, offering a lucrative off-season cash crop that complements traditional agricultural cycles (Negi et al., 2008).

However, amidst the promising prospects of pea cultivation, farmers grapple with a formidable challenge - the insidious threat of root rot. Root rot in peas manifests as a complex syndrome, characterized by a cascade of symptoms ranging from basal leaf yellowing to extensive root maceration and necrosis, ultimately culminating in the demise of affected plants. This ailment poses a significant impediment to pea cultivation, causing substantial yield losses and economic setbacks for farmers. It causes severe damage at all stages of crop growth and upto 97 per cent yield losses were reported by El-Saadony et al. (2021). In India, root rot of pea was first reported by Sukapura et al. (1957) from Pune. The magnitude of the problem is highlighted by studies elucidating the profound economic repercussions of pea root rot, accentuating the urgent need for effective disease management strategies to safeguard agricultural livelihoods.

The etiology of pea root rot is multifaceted, involving a diverse array of pathogens that inflict varying degrees of damage to pea plants. Over 20 pathogens have been implicated in the onset of root rot, including notorious culprits such as *Fusarium solani* f. sp. *pisi*, *Fusarium oxysporum* f. sp. *pisi*, *Rhizoctonia solani*, and *Phoma medicaginis* var. *pinodella*, among others (USDA 1950). These pathogens

exhibit complex interactions with host plants, triggering intricate disease processes that challenge traditional approaches to disease control (Sagar, 1996). In Himachal Pradesh, the prevalence of pea root rot has been documented, with specific pathogens such as *F. solani* f. sp. *pisi* and *F. oxysporum* f. sp. *pisi* identified as prominent causal agents of the disease complex. (Thakur et al. 2016). However, it is unclear which pathogen is exactly involved in causing the disease in Himachal Pradesh.

Despite extensive research efforts aimed at elucidating the underlying mechanisms of pea root rot, significant gaps persist in our understanding of the disease dynamics and effective management strategies. The complexity of the disease complex necessitates a comprehensive approach that encompasses morphological, cultural, and pathogenic evaluations of the implicated pathogens. By unraveling the intricacies of pea root rot and identifying key pathogenic agents, we can pave the way for the development of targeted and sustainable disease management protocols. Such protocols hold immense potential not only for mitigating the immediate economic losses associated with pea root rot but also for fostering the long-term resilience and sustainability of pea cultivation practices in Himachal Pradesh and beyond.

Keeping in view these facts we the present investigation was conducted, in which survey was conducted to record the incidence of disease at various locations of Himachal Pradesh and associated pathogen was identified on the basis of pathogenic reaction and morpho-cultural characteristics.These insights furnish pivotal guidance for the formulation of efficacious management protocols.

2 MATERIALS AND METHODS

2.1 Disease occurrence

To evaluate the prevalence of pea root rot in Himachal Pradesh, an extensive survey and surveillance initiative was undertaken across various pea cultivation regions within the state. Disease incidence data were systematically collected during the pea growing season. At each location, from every root rot effected field five random observations were conducted within 1.0 m2 quadrants to assess the disease prevalence. Plants exhibiting symptoms of root rot were carefully uprooted and placed in labelled paper envelopes, these envelopes were placed in sealable plastic bags, and stored in coolers until brought to the laboratory of the Department of Plant Pathology at Chaudhary Sarwan Kumar Himachal Pradesh Agriculture University in Palampur for further analysis. The percentage incidence of the disease was calculated using the following formula:

$$\text{Per cent Disease Incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of observed plants}} \times 100$$

2.2 Isolation and Maintenance of pure cultures

Isolations were performed using diseased samples collected from pea cultivation areas at the seedling stage to identify the pathogens responsible for pea root rot disease. The collected samples underwent initial washing in tap water followed by air-drying. Subsequently, bits were excised from the transition zone of root rot and subjected to surface sterilization that involves soaking of bits in 0.5 % sodium hypochlorite solutionand then in 70 % ethanol for 1 min each, followed by three rinses in sterilized water under aseptic conditions within a Laminar Air Flow chamber (Chittem et al. 2015).

After sterilization, the bits were air-dried on double layers of sterilized filter paper to remove excess moisture and then placed onto Potato Dextrose Agar (PDA) Petri plates under aseptic conditions. The inoculated plates were then incubated in a BOD incubator for seven days at 25±2°C. Pure cultures of the pathogens associated with the disease were obtained using the single hyphal tip method on PDA slants and subsequently preserved at 5°C in a refrigerator for further investigation. The revival of preserved pure cultures of isolates was conducted again on PDA medium.

2.2.1 Pathogenicity test

2.2.1.1 Pathogenicity test in Hoagland's solution

The rapid technique devised by Dyer and Ingram (1990) was used to conduct pathogenicity tests on the isolated pathogen(s). Seeds of the susceptible pea variety "Azad P-1" underwent surface sterilization for five minutes using a 2.5% sodium hypochlorite solution and were thoroughly washed with sterilized distilled water. These sterilized seeds were then sown in trays containing sterilized sand and irrigated with sterilized half-strength Hoagland's solution (Hoagland and Arnon, 1950).After fifteen days, pea seedlings at the 3-5 nodal stage were uprooted, and their roots were washed with sterilized water to remove any sand particles. A uniform suspension of spores, with a density of 10-12 spores per microscopic field at 10X and 40X magnification, was prepared in half-strength Hoagland's solution, referred to as the "standard inoculum".

The pea seedlings were then placed into glass tubes measuring 25mm in diameter and 150mm in length, each containing 40ml of the standard inoculum. Cotton plugs were used to hold the seedlings in place, ensuring that their roots were immersed in the spore suspension while leaving a 2cm air space between the plug and the suspension. Control seedlings were immersed in sterilized half-strength Hoagland's solution without the standard inoculum. Sterilized half-strength Hoagland's solution was replenished in the tubes every 48 hours to compensate for any loss of solution.Fifteen days post-inoculation, observations were recorded for the development of root rot, and the pathogen was subsequently re-isolated and re-inoculated to confirm its pathogenicity.

2.2.1.2 Pathogenicity test in pot

Mass multiplication of pathogens

The pure culture of *F. solani* f. sp. *pisi* was mass multiplied on sterilized sand wheat meal substrate (8:2 w/w) in conical flasks. These flasks were then incubated at a temperature of 26 ± 1°C for a duration of 15 days to facilitate mass multiplication of the fungal culture.

Preparation of sick soil

For the pathogenicity assessment, the soil underwent sterilization followed by inoculation with a wheat meal culture at a rate of 100g per kilogram of soil to induce pathogenicity. Subsequently, the inoculated soil, termed as "sick soil," was transferred into surface-sterilized plastic pots and thoroughly moistened with sterilized water. Azad P-1 pea seeds were then sown in these pots to initiate the pathogenicity test. Control pots lacking pathogen inoculum were included for comparison.

Observations were systematically conducted to monitor the development of root rot, characterized by distinct symptoms such as progressive yellowing of leaves from the basal region upwards. To confirm the causative agent of pea root rot disease, re-isolation and re-inoculation procedures were carried out. This involved isolating the suspected pathogen from affected plants, culturing it, and subsequently

reintroducing it to healthy plants to observe for symptom reproduction, thereby confirming its role in the disease.

2.3 Pathogen identification (Based on Morpho-cultural characteristics)

Microscopic examinations were conducted to observe the morpho-cultural characteristics of the isolated pure culture of the pathogen, with its taxonomic classification confirmed in accordance with the keys Booth (1975) given below:

List 1 : Taxonomic classification in accordance with the keys Booth (1975)

Parameter	*Fusarium solani*
Colour of colony on PDA	White to cream colonies growing rapidly with aerial mycelium becoming bluish-brown when sporodochia formed
Growth on PDA	4.5 cm in four days
Macroconidia	Macroconidia of 28-42 x 4-6 µm with three to five septa and fusiform, cylindrical, often moderately curved, with an indistinct pedicellate at foot cell and a short blunt apical point.
Microconidia	Cylindrical to oval micro conidia of 8-16 x 2-4.5 µm with one to two-celled are abundantly present.
Chlamydospores	Smooth to rough-walled chlamydospores of 6-10 µm borne singly or in pairs on short lateral hyphal branches or intercalary

a) Morphological

The morphological characteristics of the pathogen, including shape and dimensions of its conidia (both macroscopic and microscopic) and chlamydospores, were scrutinized utilizing a compound microscope at magnifications of 10X and 40X.

b) Cultural characteristics

Mycelial bits measuring 5 mm in diameter were excised using a sterilized cork borer and subsequently placed at the central region of Petri dishes containing Potato Dextrose Agar (PDA). The plates were then incubated at a temperature of 26±1°C within a BOD (Biological Oxygen Demand) incubator. Observations pertaining to colony growth and pigmentation were recorded from seven days old cultures.

3 RESULTS AND DISCUSSION

3.1 Occurrence of pea root rot disease

In recent observations, pea root rot has emerged as a serious concern within select pea cultivation regions of Himachal Pradesh. Across various districts, differing levels of disease incidence have been recorded, as detailed in Table 1. The highest disease incidence, reaching 75.2%, was observed in Bir, followed closely by Chougan at 74.1%, and Kukumseri at 73.9%. Subsequent locations also reported varying degrees of incidence, including Lahar (73.1%), Gunehar (72.3%), Sagoor (71.4%), Paprola (70.2%), Sungal (69.6%), and Baijnath (Girtholi) at 69.5%. Lower but still notable rates were recorded in Dhraman (63.5%), Nagrota (63.4%), Banuri (61.3%), Chauntra (60.9%), Joginder Nagar (57.8%), the Organic Farm at CSK HPKV Palampur (56.7%), Bajaura (56.0%), Nagwain (54.8%), and Malan (54.5%). The Plant Pathology Farm at CSK HPKV Palampur documented a disease incidence of 50.2%.

Table 1 Incidence of root rot of pea in different locations

Sr. No.	Location	Latitude (°N)	Longitude (° E)	District	Incidence (%)
1	Plant Pathology Farm,	32.1109	76.5363	Kangra	50.2

2	CSKHPKV Palampur Organic Farm, CSKHPKV Palampur	32.1109	76.5363	Kangra	56.7
3	Banuri	32.1018	76.5575	Kangra	61.3
4	Dhraman	31.9886	76.7120	Kangra	63.5
5	Sungal	32.0835	76.5821	Kangra	69.6
6	Pathiarkhar	32.0919	76.5886	Kangra	65.1
7	Paprola	32.0528°	76.6341	Kangra	70.2
8	Sagoor	32.0339	76.6138	Kangra	71.4
9	Baijnath (Girtholi)	32.0401	76.6519	Kangra	69.5
10	Bir	32.0456	76.7236	Kangra	75.2
11	Chougan	32.0116	76.4838	Kangra	74.1
12	Lahar	32.1406	76.1103	Kangra	73.1
13	Gunehar	31.8618	76.4568	Kangra	72.3
14	Nagrota	32.1054	76.3789	Kangra	63.4
15	Malan	32.0866	76.3502	Kangra	54.5
16	Joginder Nagar	31.9912	76.7899	Mandi	57.8
17	Chauntra	32.0138	76.7495	Mandi	60.9
18	Nagwain	31.8150	77.1801	Mandi	54.8
19	Bajaura	31.8465	77.1605	Kullu	56.0
20	Kukumseri	32.6990	76.6882	Lahaul and Spiti	73.9

The findings depicted in Plate 1 corroborate the incidence data, highlighting the widespread presence of pea root rot across diverse pea cultivation regions. The highest incidence of 75.0 per cent was reported from Bir due to *F. solani*(Sagar1996). Thakur et al. (2016) also recorded the incidence of pea root rot at many places in Himachal Pradesh and found that the disease had assumed a severe form with incidence of 54.7 per cent at Kukumseri followed by Palampur (35.3%).As depicted from results it was seen clearly seen that was very high diversity in incidence levels of disease at different locations. The difference in incidence can be of various factors. As regions experiencing specific environmental conditions, such as high humidity, excessive moisture or different cropping patterns like monoculture of peas increasing the likelihood of disease occurrence and severity.Pathogen Persistencein the soil or presence of more or less virulent races of *Fusarium solani* f.sp. *pisi* lead to different disease incidence levels at different regions. These factors highlights the need of further research work for mitigating the high incidence of pea root rot in affected locations.

Plate 1. Occurrence of pea root rot in farmers fields

3.2 Identification of pathogen(s)

a) Isolation of Pathogen(s) and maintenance of pure cultures

Disease samples exhibiting symptoms of pea root rot were systematically collected from various pea cultivation regions across the state during extensive survey and surveillance efforts. Isolations of the pathogen were conducted utilizing these collected samples. Pure cultures of the pathogen were successfully obtained using the single hyphal tip method. These cultures were subsequently maintained on Potato Dextrose Agar (PDA) medium at a controlled temperature of 26 ± 1°C to facilitate further comprehensive studies.

a) Morphological, cultural and pathogenic characteristics

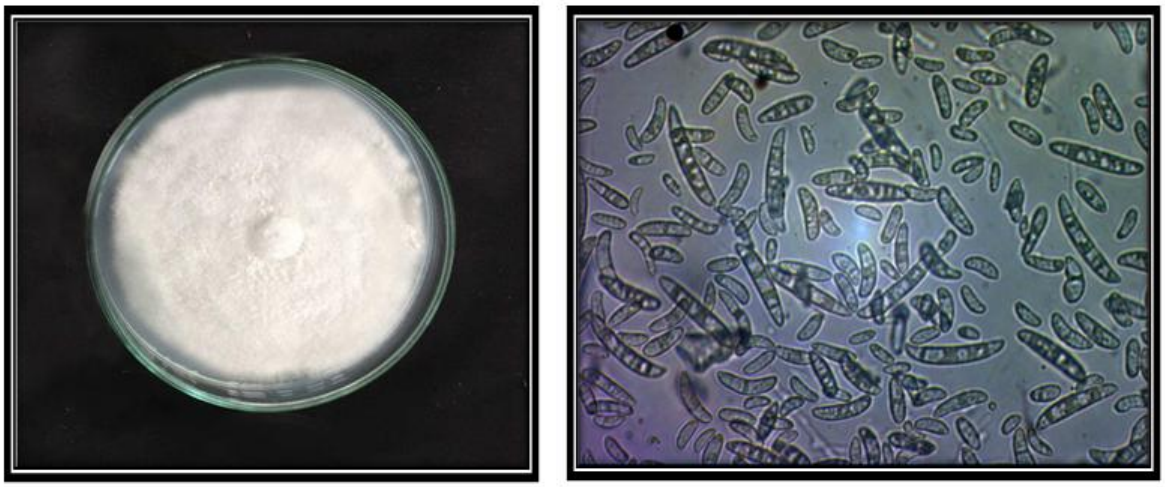
The pure culture of the isolated pathogen underwent comprehensive morphological, cultural, and pathogenic evaluation, as outlined in Table 2. Identification of the pathogen was done using established keys given by Booth (1975). Morphologically, the mycelium exhibited a dull white (creamy) color, and the macroconidia showed minimal curvature, possessing 3-5 septa with dimensions measuring 30-40 x 4.5-6.0 µm. Microconidia appeared spherical to oval, measuring 8.0-15 x 2.0-4.0 µm (Plate 2). Rough-walled chlamydospores, ranging from 6-9 µm, were observed on hyphal branches or intercalary positions. Pathogenicity assessments were performed using Hoagland's solution in test tubes (Plate 3a) and pot cultures (Plate 3b). Characteristic symptoms of root rot, initially manifesting as brownish macerated root tissues extending to the collar region, progressed to necrosis. Affected plants showed yellowing from the basal leaves upwards. Based on the collective morphological, cultural, and pathogenic characteristics, the pathogen associated with pea root rot was conclusively identified as *Fusarium solani* f. sp. *pisi.*

Table 2 Pathogen identification**:**

Keys for identification		**Pathogen associated with pea root rot**								
Parameter	***Fusarium solani***	Isolate	Colour of colony on PDA	Growth on PDA	Macroconidia	Microconidia	Chlamydospores	*Pathogenicity	Symptom	Pathogen identified
Colour of colony on PDA	White to cream colonies growing rapidly with aerial mycelium becoming bluish-brown when sporodochia formed.	Pea root rot	Dull white (creamy) cottony colour mycelium	4.5 cm in four days	Macro conidia were not sharply curved having 3-5 septa and measured 30-40 X 4.5-6.0 µm	Micro conidia were spherical to oval in shape and measured 8.0-15 x 2.0-4.0 µm	Rough walled chlamydospores of 6-9 µm were found on hyphal branches or intercalary	+	Initially brownish macerated root tissues (root rots) extends upto collar region and later on turns necrotic. Affected plants show yellowing from basal leaf to upward.	*Fusarium solani* f. sp. *pisi*
Growth on PDA	4.5 cm in four days									
Macroconidia	Macroconidia of 28-42 x 4-6 µm with three to five-septa and fusiform, cylindrical, often moderately curved, with an indistinct pedicellate at foot cell and a short blunt apical point.									
Microconidia	Cylindrical to oval micro conidia of 8-16 x 2-4.5 µm with one to two-celled are abundantly present.									
Chlamydospores	Smooth to rough-walled chlamydospores of 6-10 µm borne singly or in pairs on short lateral hyphal branches or intercalary									

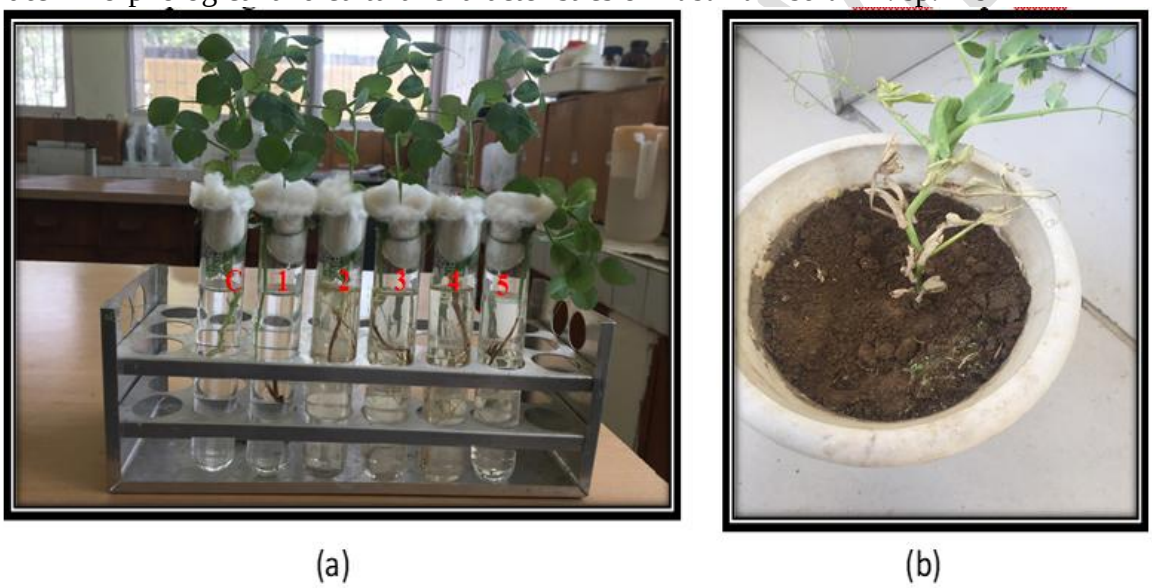
+ = Pathogenic

- = Non-pathogenic (Booth 1975)



Culture of *Fusarium solani* f.sp. *pisi*. Microscopic images of *Fusarium solani* f.sp. *pisi*.

Plate 2 Morphological and cultural characteristics of *Fusarium solani* f. sp. *Pisi*



(a) (b)

Plate 3 Pathogenicity tests of *Fusarium solani* f. sp. *pisi*:(a) In Hoagland's solution and (b) in pot culture

The research conducted by Dohroo et al. (1998) and Thakur et al. (2016) provides substantial evidence supporting the identification of *Fusarium solani* f. sp. *pisi* as the primary causative agent of pea root rot in Himachal Pradesh. The consistent identification of *Fusarium solani* f.sp. *pisi* by both research teams shows the reliability of the conclusion. The fact that two independent studies conducted at different times have arrived at the same conclusion strengthens the confidence in the identification of the pathogen.

Hence, *F. solani* f. sp. *pisi* is confirmed to be the causal agent of pea root rot in Himachal Pradesh.

4 CONCLUSION

In conclusion, *Fusarium solani* f. sp. *pisi* has been identified as the primary causative agent of pea root rot in Himachal Pradesh, with alarming incidence rates observed across various districts. Given the severity of pea root rot in Himachal Pradesh, it is imperative to implement effective management strategies to mitigate its impact on pea cultivation.While the identification of the causal agent of pea root rot represents a significant advancement in understanding the disease, there are several aspects for future research to explore like

investigating the genetic diversity of *Fusarium solani* populationsin different pea cultivation regions and studying the molecular mechanisms underlying the interaction between the pathogen and pea plants to identify key genes involved in disease resistance and susceptibility, with the aim of developing resistant cultivars through breeding programs or genetic engineering.

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