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

Abstract

The present investigation had been undertaken in the Department of Plant Pathology, College of Agriculture, Chaudhary Sarwan Kumar Himachal Pradesh Agriculture University, Palampur (India)during 2017-2019. Pea root rot had been observed as an alarming problem in pea growing regions of Himachal Pradesh and poses major constraints in pea cultivations. At Bir, the highest disease incidence of 75.2 per cent was recorded. The disease occurs in complex as pea root rot/wilt complex. More than 20 pathogens have been reported to be associated with the disease from different parts of the world. In Himachal Pradesh, *Fusarium solani* f. sp. *pisi* was found to be associated with pea root rot in the state. The pathogen producedcharacteristic symptoms in pathogenicity tests as blackening and maceration of pea roots resulted in yellowing of leaves from basal leaf to upward.

Keywords: Root rot, Incidence, *Fusarium solani*

1 INTRODUCTION:

Pea (*Pisum sativum* L.) stands as a prominent cash crop thriving predominantly in cooler regions worldwide. Valued both as a vegetable (green peas) and a pulse (dried peas), it serves as a lucrative off-season cash crop, particularly in regions such as Lahaul and Spiti, Kinnaur, Shimla, Kullu, Mandi, and Kangra in the state, as noted by Bhardwaj and Vikram (2004). However, the cultivation of peas faces a significant threat from root rot, an emerging and severe ailment that initiates with basal leaf yellowing, leading to root maceration, necrosis, and ultimately, the demise of affected plants. This affliction inflicts substantial crop losses, thereby imposing considerable economic burdens on farming communities (Negi et al., 2008).

The root rot disease in peas has been attributed to more than 20 pathogens, including *Fusarium solani* f. sp. *pisi*, *Fusarium oxysporum* f. sp. *pisi*, *Rhizoctonia solani*, *Phoma medicaginis* var. *pinodella*, *Mycosphaerella pinodes*, *Aphanomyces euteiches*, *Thielaviopsis basicola*, and various species of *Pythium* such as P. ultimum, *P. debaryanum*, *P. vexans*, *P. splendens*, *P. aphanidermatum*, and *P. irregulare*, as documented by USDA (1960). In Himachal Pradesh, the reported pathogens include *F. solani* f. sp. *pisi*, *F. oxysporum* f. sp. *pisi*, *R. solani, and P. medicaginis* var. *pinodella* (Sagar, 1996). Recent investigations by Thakur et al. (2016) have specifically identified two *Fusarium* species, *F. solani* f. sp. *pisi* and *F. oxysporum* f. sp. *pisi*, as causal agents of pea root rot and wilt complex disease.

This scientific exposition emphasizes the intricate complexity of pea root rot, highlighting its profound economic ramifications and delineating the precise pathogens implicated in its onset. 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, five random observations were conducted within 1.0 m2 quadrants to assess the disease prevalence. Plants exhibiting symptoms

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-study's scale and sample, **quantification**of the economic impact of pea root rot, **Context**regarding the discovery of Fusarium solani f. sp. pisi and its virulence compared to other pathogens.**Clarification** on the diseases included in the pea root rot/wilt complex.**Global significance** of the local findings in the context of worldwide research.**Methodological details** of the pathogenicity tests, including controls and setup.

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- A more detailed literature review, a clearer statement of the research gap and objectives, a brief methodological overview, and a highlight of the significance and practical implications of the findings.

Comment [s4]: Please try to address the following issues on your methodology;
i-The study relies on only five random observations within 1.0 m² quadrants, which may not provide a comprehensive representation of the disease prevalence across different regions.
ii-Preserving diseased plants in paper envelopes for transport risks further spread of the disease and may compromise the integrity of the samples.
ii-brief 15-second sterilization using a 1.0% sodium hypochlorite solution followed by rinsing may not be sufficient to eliminate all contaminants, potentially affecting the purity of the cultures.
ii-The specific incubation conditions (26±1°C) in the BOD incubator may not be optimal for all pathogens, possibly leading to selective growth and not reflecting the full diversity of the disease-causing agents.

of root rot were carefully uprooted and preserved in labelled paper envelopes, which were subsequently transported to the Department of Plant Pathology laboratory 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 using a 1.0 % sodium hypochlorite solution for 15 seconds, followed by three rinses in sterilized water under aseptic conditions within a Laminar Air Flow chamber.

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 26±1°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

Comment [s5]: pleaseprovide relevant informationthe following issues,
-**High Incidence Rates,**the study reports extremely high disease incidence in certain locations, with the highest being **75.2%** in Bir.why ?
-**Variability Across Regions,**There is significant variability in disease incidence across different districts,I gues thereareadditonal fatorscontribute tovariabltyor errorindata collection or management.
-**Need for Further Research,** the results underscore the necessity for further research to understand the causes of such high incidence rates and to develop effective control measures, why?

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, CSKHPKV Palampur	32.1109	76.5363	Kangra	50.2
2	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. Manifesting with severity, the disease prevalence reached alarming levels in numerous locations. Notably, Bir reported the highest incidence at 75.0%, due to*F. solani* as documented by Sagar in 1996. Thakur et al. (2016) similarly documented significant occurrences of pea root rot throughout Himachal Pradesh. Particularly noteworthy was the severe manifestation observed, with an incidence rate of 54.7% in Kukumseri, followed by Palampur at 35.3%.

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.

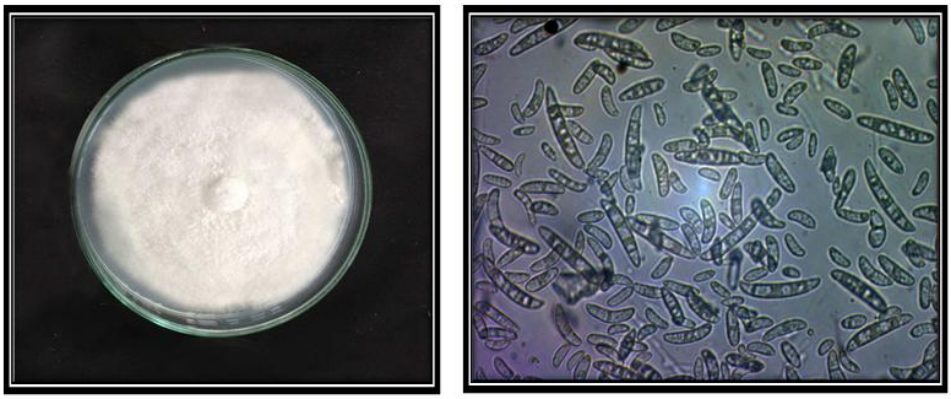
a) Morphological, cultural and pathogenic characteristics

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

Root rot of pea has been observed as an emerging and alarming problem in pea growing regions of Himachal Pradesh.*F. solani* f. sp. *pisi* was found to be associated with root rot of pea in Himachal Pradesh.

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