

# Incidence, Phytopathological effects and disease transmission of leaf spot disease of pea in Rajasthan, India

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**Abstract:** Pea (*Pisum sativum* L.) is an important leguminous seed crop grown for vegetable and food purpose. Leaf spot disease of pea caused by *Xanthomonas campestris* pv. *pisi* is a threat for the production, yield and quality of seeds. During field surveys, pea seeds were collected from major pea growing districts of Rajasthan. The collected seeds upon incubation yielded bacterial pathogen identified as *Xanthomonas campestris* pv. *pisi* by biochemical and molecular characterization using 16s rRNA sequencing. The pathogen was detected in one hundred and nineteen seed samples on YDC agar medium with 3-89.5% incidence from different districts of Rajasthan. To study the phytopathological effects and seed to seedling disease transmission three different methods viz. Petri plate method, water agar test tube symptom test and pot experiment were used. In Petri plate method and test tube seedling symptom test, the pathogen observed as white to pale cream oozing on and around the seeds. The pathogen caused the symptoms initiated as irregular brown to black spots of radical and plumule which later showed rotting. Symptomatic seedlings showed bacterial oozing, brown irregular water-soaked spots and browning of cotyledonary leaves which later showed blighted collapsed hypocotyls. Loss of germination and mortality of seedlings were found higher in shrivelled discoloured seeds. The pre- and post emergence losses recorded due to pathogens studied.

**Keywords:** Pea seeds, *Xanthomonas campestris* pv. *Pis*, Seedling symptoms, transmission.

## Introduction

Pea (*Pisum sativum* L.) belongs to family fabaceae and is an important vegetable crop grown throughout the world. Like other pulse crops, diseases caused by fungi, bacteria, viruses and nematodes are among the notable risk factors of pea cultivations. Peas are mainly utilized as a vegetable. Besides, it is also consumed as pulse. Sometimes, the field pea is grown for forage and green manure and the pods are sometimes fed to farm animals. Leaf spot disease of pea caused by *Xanthomonas campestris* pv. *pisi* is a seed-borne bacterial pathogen. (Neergard, 1977, Richardson, 1990, Garden *et al.* 1999). In the fields, the plants showed various disease symptoms of circular to irregular brown spots on the leaves showing leaf spot disease. In severe cases the complete defoliation and petiole browning were observed. In the present study, the phytopathological effects and seed to seedling disease transmission leaf spot disease pathogen on pea crop was determined.

## Materials and methods

Two seed samples each of naturally infected with *Xanthomonas campestris* pv. *pisi* (acc. nos. Xp 2528 and Xp-2542 with 85 and 89.5% incidence respectively) were used for the study. The study was carried out by using following method:

(i) Petri plate method, (ii) Water agar test tube seedling symptom test and (iii) Pot experiment

The percentage of seed germination, ungerminated seeds associated with the pathogen (oozing), symptomatic seedlings and mortality of seedlings were recorded. **(i) Petri plate method**

The seeds were pretreated with aqueous solution of hypochlorite solution with 2% available chlorine and placed on moistened blotters in Petri plates with three replicates of 100 seeds per sample per category. The Petri plates were incubated at 25±2°C under 12/12h alternating cycles of day light and darkness for 8 days. Observations were recorded daily upto 8 days.

**(ii) Test tube seedlings symptoms test**

Three replicates of 100 seeds per category per sample were sown on 1% sterilized water agar medium in test tube (1 seed/test tube) under aseptic condition and incubated at 25±2°C for 12/12h alternating cycles of light and darkness. Observation on seed germination, seedlings symptoms and seedlings mortality were recorded upto 15 days.

**(iii) Pot experiment**

One hundred seeds per category per sample (5 seeds/pot) were sown in earthen pots containing sterilized soil. All the pots were watered on every alternate day. Observations for pre and post emergence losses, disease symptoms on seedlings and/or plants and mortality were recorded upto fruit setting at weekly intervals. To isolate the pathogen associated with symptomatic seedlings and plants, symptomatic parts were harvested, washed and sterilized with 2% available chlorine and incubated on moistened blotters for 7 days and also on nutrient agar (NA) medium to isolate the pathogen(s) associated. After the pot experiment, pods were harvested and obtained seeds were categorized and the pathogen was reisolated.

## Results and discussion

### Field observation

In the fields, the plants showed various disease symptoms of circular to irregular brown spots on the leaves showing leaf spot disease. In severe cases the complete defoliation and petiole browning were observed. The small and large spots on leaves coalesced and showed necrosis. Brown lesions and streaks were observed on the stems. At fruiting stage browning and rotting of pods were observed. On incubation all the infected plant parts and pods yielded the pathogen.

### Experimental studies

Two seed samples of pea naturally infected with *Xanthomonas campestris* pv. *pisi* (acc. nos. Xp-2528 and Xp-2542 with 85% and 89.5% incidence respectively) were selected for transmission studies.

#### 1. Petri plate method

The seed germination started after 24 hr of incubation and on the 6<sup>th</sup> day it was maximum to be 96 and 94% in asymptomatic, 88 and 86% in moderately discoloured and 71 and 78% in shrivelled discoloured seeds in acc. no. Xp-2528 and Xp-2542 respectively. The ungerminated seeds were 4, 12 and 29% in acc. no. Xp-2528 and 6, 14 and 22% in acc. no. Xp-2542 in the three seed categories respectively. The symptomatic seedlings were 66.31, 85.91 and 90.36% in acc. no. Xp-2528 and 58.36, 79.31 and 88% in acc. no. Xp-2542 in the three categories respectively. The initial symptoms were recorded at root-shoot transition zone. The cotyledonary leaves showed rotting with etiolation. The seedling mortality was 3.19, 10 and 29.33% in acc. no. Xp-2528 and 2.11, 13 and 27.61% in acc. no. Xp-2542 in the three categories respectively. The total loss (pre and post emergence) due to the pathogen was 9, 15 and 74% in acc. no. Xp-2528 and 8, 23 and 77% in acc. no. Xp-2542 in the three categories respectively.

#### 2. Water agar test tube seedling symptoms tests (TTSST)

Radical emergence started after 24h of incubation the seed germination was 90, 87 and 49% in acc. no. Xp-2528 and 92, 87 and 56% in acc. no. Xp-2542 in three categories respectively on 15<sup>th</sup> days of incubation. The shrivelled discoloured seeds failed to germinate due to the heavy oozing of the pathogen on and around the seeds. The percentage of such seeds was 10, 13 and 51% in acc. no. Xp-2528 and 8, 13 and 44% in acc. no. Xp-2542 in the three categories respectively. The symptoms appeared on 5<sup>th</sup> day of seed germination and continued up to 15<sup>th</sup> day. The symptomatic seedlings showed browning at cotyledonary leaves, root-shoot transition and on root tip, in few seedlings where only radicle emergence occurred, puffing and rotting was observed. The symptomatic seedlings were 22, 14.64 and 82.63% in acc. no. Xp-2528 while in acc. no. Xp-2542 was 2.18, 16.64 and 88% in the three categories respectively. The mortality was 13.33, 16 and 67.35% in acc. no. Xp-2528 and 12, 18.35 and 63% in acc. no. Xp-2542 in three categories respectively. The total loss (pre and post emergence) due to the pathogen was 20, 23 and 78% in acc. no. Xp-2528 and 19, 28 and 69% in acc. no. Xp-2542 in three categories respectively.

#### 3. Pot experiment

The appearance of symptoms and seed emergence varied according to the seed category. In asymptomatic category seed germination started after 5<sup>th</sup> days whereas in moderately discoloured and shrivelled discoloured categories it took 15-20 days. Most of the shrivelled discoloured seeds failed to germinate due to heavy growth of the pathogen. The symptoms started appear after 25-30 days of sowing in moderately discoloured seeds. In shrivelled discoloured category the symptoms appeared on 15-20<sup>th</sup> days of sowing. Browning, small, irregular spots on leaves and browning of transition zone of seedlings were observed. The spots on leaves coalesced and formed large spots, blighting and wilting symptoms on them in severe conditions. The heavily infected seedlings dried and collapsed after 30 days of seed germination. The total loss was 9, 15 and 66% in acc. no. Xp-2528 and 8, 16 and 64% in acc. no. Xp-2542 in asymptomatic, moderately discoloured and shrivelled discoloured seeds respectively. The infected plants developed from surviving seedlings were usually weak and the leaves and pods were smaller than the healthy plants (Fig. 1).

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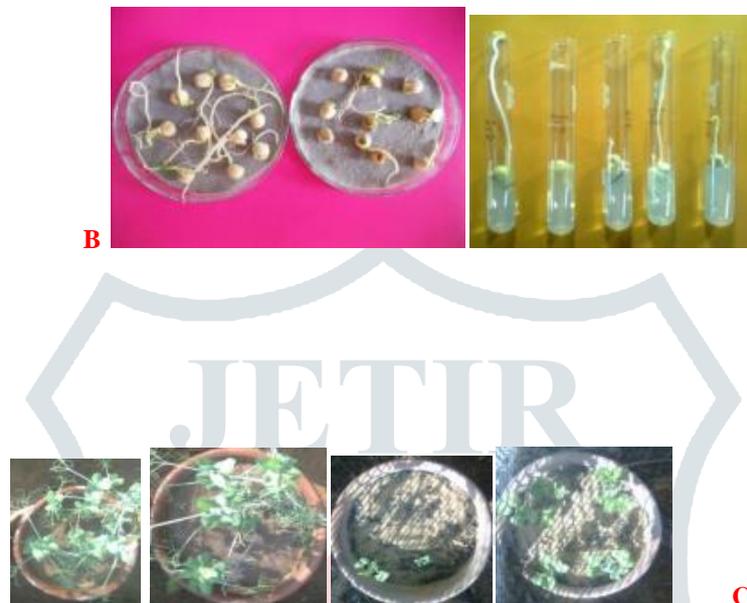


Fig. 1. Study of phytopathological effects and disease transmission of leaf spot disease of pea using Petri plate and Pot experiments