



# Plant Pathology Journal

ISSN 1812-5387

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## Pathological Studies on *Alternaria alternata* (Fr.) Keiss. Causing Leaf Blight of Pear

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**Abstract:** In present study strong pathogenic capability of the fungus *Alternaria alternata* was established on pear leaves. The bit placement method was adjudged superior to drop placement and spray inoculation as percent recovery of the disease was higher in bit placement inoculation. The four test isolates exhibited variation in extent of sporulation, pathogenic variability and growth pattern. Bhoonga (Hoshiarpur) isolate was found to be most virulent followed by PAU (Ludhiana) and Attari (Amritsar). None of the four test varieties was found to be resistant or moderately resistant under natural conditions as well as under *in vitro* and *in vivo* inoculation conditions. Screening under both *in vitro* and field conditions showed that Punjab Gold was moderately susceptible to the disease while Patharnakh and Punjab Beauty were susceptible and Punjab Nectar was highly susceptible. It was found that the pathogen survived on the infected fallen leaves, which help in its perennation.

**Key words:** Pathological studies, *Alternaria alternata*, leaf blight, pear

### INTRODUCTION

The genus *Alternaria* contains ubiquitous species of fungi, including aggressive and opportunistic plant pathogens affecting the majority of cultivated crops. One of the best-known and economically important members of the genus is *Alternaria alternata* (Fr.) Keiss. Pathogenic *Alternaria alternata* was recorded to infect pear for the first time on cultivar Nijisseiki in Japan<sup>[1]</sup>. In India, pear was first reported to be infected by *Alternaria alternata* by Koul and Narain<sup>[2]</sup> from Wadoora, Kashmir. The disease has also been reported from Korea<sup>[1]</sup>, Greece<sup>[3]</sup>, Italy<sup>[4]</sup> and France<sup>[5]</sup>. Recently, disease has been noticed in Punjab with incidence varying from 48 to 54% in major pear-growing areas<sup>[6]</sup>. As *Alternaria* is generally considered to be a facultative or saprophytic type of organism, hence efforts were made to confirm pathogenicity, study variability in test isolates with respect to pathogenic potential, growth pattern and sporulation, screen different pear varieties for resistance to this serious disease and study perpetuation of the pathogen.

### MATERIALS AND METHODS

Surveys of pear plantations were conducted at New Orchard, Punjab Agricultural University, Ludhiana, Govt. orchard at Attari (Amritsar) and Bhoonga (Hoshiarpur) in the second fortnight of August. Isolations from the diseased leaves were carried out on Potato Dextrose Agar

(PDA) slants using standard method and isolates obtained were studied for extent of sporulation, growth pattern and pathogenic variability in terms of lesion size and Percent Disease Index (PDI) after 10 days of inoculation.

Healthy leaves were collected from pear variety Punjab Nectar during July 2000 from New Orchard, Punjab Agricultural University, Ludhiana washed thoroughly under running tap water and air-dried. Inoculations with the fungus culture derived from the infected pear leaves were carried out using three methods i.e., culture bit placement (8 mm culture bit placed on leaves), inoculum drop placement (containing  $4 \times 10^5$  spores/mL) and atomizing inoculation methods (spore suspension containing  $4 \times 10^5$  spores/mL). Under each type of inoculation method, three sets of leaves were maintained: injured leaves, uninjured leaves and control. In case of injured leaves shallow circular injury of approximately 5 mm diameter was inflicted on leaves using refill-pen tip that was sterilized by heating on the flame of spirit lamp. The inoculation methods were evaluated in terms of time required for expression of symptoms as well as number of lesions produced after 10 days of inoculation.

To screen varieties against the disease, severity of the disease under field conditions on the four varieties was recorded in terms of percent disease index (0-4 scale) using the formula given by Mc Kinney<sup>[7]</sup> in the last week of August 2000. Then, the varieties were screened for resistance to the disease under artificial inoculation conditions *in vitro* and *in vivo*. Detached leaves of

uniform size and similar age group from each of the four cultivars were collected and inoculated *in vitro*. Data on PDI were recorded after 10 days of inoculation. Leaves of test varieties were also inoculated under field conditions. On the basis of PDI, the pear cultivars were categorized as resistant, R (PDI = 0-5%); moderately resistant, MR (PDI = 6-10%); moderately susceptible, M.S (PDI = 11-20%); susceptible, S (PDI = 21-50%) and highly susceptible, HS (PDI > 50%).

Naturally infected pear leaves were collected from the New Orchard, Punjab Agricultural University, Ludhiana in the mid of October 2000 and kept protected in wire gauge baskets in the field and in polythene bags in the laboratory at room temperature. Periodical isolations from these leaves were carried out on Potato Dextrose Agar (PDA) slants after every 30 days to ascertain the longevity of fungus in infected leaves upto June 2001. Observations in terms of percent recovery of the pathogen on PDA were recorded.

### RESULTS AND DISCUSSION

As presented in Fig. 1 in bit placement inoculation fungus could incite that blight disease successfully on cent percent inoculation sites on the leaves with a shallow surface injury and symptoms started appearing after 48 h of inoculation. On uninjured leaves, by the same method 92% inoculation sites resulted into typical blight lesions and symptoms started appearing after 120 h of inoculation. The blight disease appeared on 97% inoculation sites with a shallow surface injury when inoculations were carried out by drop placement method. Symptoms started appearing on these leaves after 48 h of inoculation. However, on the uninjured leaves, 65% inoculations sites could be successfully infected with the fungus by the same method and symptoms started appearing on these leaves after 120 h of inoculation.

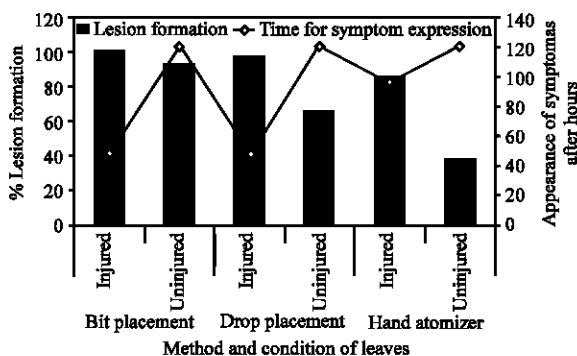


Fig. 1: Pathogenicity tests of *Alternaria alternata* on detached leaves of pear cultivar Punjab Nectar

Comparatively lesser number of inoculation sites (85% on injured and 38% on uninjured leaves) could results into typical blight symptoms when inoculations were carried out by spraying the spore suspension using hand atomizer. Time required for the appearance of symptoms on injured leaves by atomizing method was more as compared to the rest of the two methods. The observations clearly established the strong pathogenic capability of the fungus on pear leaves. The bit placement method was adjudged superior to drop placement and spray inoculation methods in present studies as per cent recovery of the disease was higher in bit placement inoculation.

I<sub>1</sub> (Bhoonga) isolate was found to be most virulent forming lesions of the size of 7.06 mm with PDI to the tune of 53.87 which was followed by I<sub>2</sub> (PAU) isolate (5.41 mm and 51.52%) I<sub>3</sub> (Attari) isolate was found to be least virulent amongst three isolates forming the lesions of the size of 3.15 mm and registering PDI of 47.61 (Table 1).

None of the four varieties was found to be resistant or moderately resistant under natural conditions (i.e., without inoculations). Punjab Gold expressed moderately susceptible reaction whereas Patharnakh and Punjab Beauty showed highly susceptible reaction (Table 2). Out of the four cultivars screened under *in vitro* conditions by inoculating the detached leaves, Punjab Gold was found to be moderately susceptible to the disease. Patharnakh and Punjab Beauty showed susceptible reaction while Punjab Nectar was found to be highly susceptible (Table 3). Inoculations under field

Table 1: Pathogenic and morphological variability of isolates obtained from different regions of Punjab

Isolate*	Extent of sporulation (conidia/msf)**	Average size of lesion (mm)***	PDI	Colony characteristics
I <sub>1</sub>	86	7.06	53.87	Colony raised, ring pattern very prominent
I <sub>2</sub>	79	5.41	51.52	Colony slightly raised, ring pattern less prominent
I <sub>3</sub>	72	3.15	47.61	Colony flat, ring pattern least prominent

\* I<sub>1</sub>=Bhoonga isolate, I<sub>2</sub>=PAU isolate, I<sub>3</sub>= Attari isolate

\*\* Based on five microscopic fields

\*\*\*Based on culture bit placement method, Average of 100 lesions

Table 2: Occurrence of *Alternaria* leaf blight on different pear cultivars under field conditions naturally

Cultivar	Percent disease index (PDI)*	Reaction
Punjab Nectar	52.92	HS
Punjab Beauty	46.64	S
Patharnakh	27.32	S
Punjab Gold	19.45	MS

R= Resistant (PDI= 0-5%); T = Tolerant (PDI = 6-10%); MS = Moderately susceptible (PDI= 11-20%); S = Susceptible (PDI=21-50%); HS = Highly susceptible (PDI>50%), \* Observations recorded in last week of August

conditions on standing plants confirmed that Punjab Gold was moderately susceptible showing a percent disease index of 13.37 and symptoms started appearing after 96 h of inoculation whereas on Patharnakh although the time taken for the appearance of symptoms was the same as that Punjab Gold but per cent disease in index was found to be comparatively higher (22.13) thus rated as susceptible. Punjab Beauty showed susceptible response with percent disease index of 38.52 and symptoms appeared after 72 h of inoculation. Punjab Nectar was found to be highly susceptible (50.90) and symptoms appeared after only 48 h of inoculation (Table 4).

Periodical isolations revealed that pathogen could successfully survive on infected pear leaves kept protected under field conditions as well as at room

temperature. There was 100% recovery of the pathogen up to 150 days (after mid of October) in case of infected pear leaves kept at room temperature whereas 100% recovery up to 90 days was observed in case of infected pear leaves kept protected under field conditions. Even after 240 days i.e. up to June 2001, which is the time for the onset of the disease in the field, the recovery from the infected leaves kept protected under field conditions and those kept at room temperature was to the extent of 66.6 and 86.6%, respectively (Fig. 2). It was found that the pathogen survived on the infected fallen leaves and which help in its perennation. These observations are in agreement with that of Nam and Kim<sup>[8]</sup>.

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Table 3: Relative susceptibility of different pear cultivars against *Alternaria alternata* on detached leaves

Cultivar	Percent disease index (PDI)*	Reaction
Punjab Nectar	51.60	HS
Punjab Beauty	41.47	S
Patharnakh	25.87	S
Punjab Gold	15.87	MS

R= Resistant (PDI= 0-5%); T =Tolerant (PDI = 6-10%); MS = Moderately susceptible (PDI= 11-20%); S = Susceptible (PDI=21-50%); HS = Highly susceptible (PDI>50%), \* After 10 days of inoculation

Table 4: Relative susceptibility of different pear cultivars against *Alternaria alternata* inoculated artificially under field conditions

Cultivar	Incubation period (h)	Percent disease index (PDI)*	Reaction
Punjab Nectar	48	50.90	HS
Punjab Beauty	72	38.52	S
Patharnakh	96	22.13	S
Punjab Gold	96	13.37	MS

R= Resistant (PDI= 0-5%); T =Tolerant (PDI = 6-10%); MS = Moderately susceptible (PDI= 11-20%); S = Susceptible (PDI=21-50%); HS = Highly susceptible (PDI>50%), \* After 10 days of inoculation

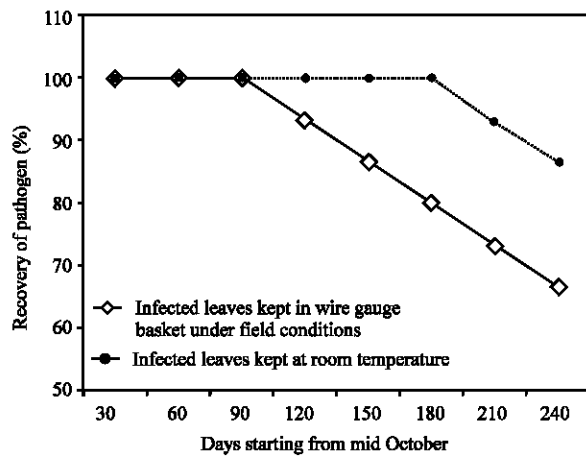


Fig. 2: Perpetuation of *Alternaria alternata* causing leaf blight of pear