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## TO STUDY OF ISOLATION AND IDENTIFICATION OF PATHOGEN CAUSING LEAF AND BUD SPOT DISEASE OF LINSEED

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### Abstract

. The *Alternaria lini*, which cause a brown and black target like lesion on the leaves and buds at the binging stage and can cover the whole leaf surface lead to curled and dried of leaves and bud of linseed causing failure of flower to open during the day resulting failure of oval development for seed setting and falling of dried buds after touching by hand or even with speedy wind. Infected capsule did not bear the seeds and at last their capsules were disturbed blighted discoloured and undeveloped The hyphae were Septate, branched, hyaline, colourless turned pale then olive grey. The mycelial width was 3.0 – 5.3 µm. Conidiophores are septate, erect, branching or non-branching geniculate, olive buff to dark olive buff, 21.5 – 100.5 µm wide.

**Key words:** *Linseed, Alternaria lini, isolation & identification, Pathogen, Bud Disease*

### Introduction

This study was carried out at the research farm of BNPG College Rath, Hamirpur, Uttar Pradesh, India during *Rabi* season in the two successive years, *i.e.* 2013-14 and 2014-15 and survey was carried out to get the objective from different villages namely Dhamna, Sarsai, Basela, from block Rath, and Bragarh, Rahank from block Gohand of district Hamirpur, UP, for collection of sample of disease. Analization of random samples was carried out in the laboratory of pathology at BNPG College Rath, Hamirpur.

As Rath is on a sub soil plateau, the temperature here is extreme. Winters starts in October with end of monsoon and on peak in mid December. The average temperature of winter lies between 6 degrees at night and 24 degrees in the day on the Celsius scale. Spring seasons ends in February, making the ends of winter. Summer starts in April where the temperature lies between 34 and 46degree Celsius in the Day. However, the night in the summer is cool after mid night. The third week in June brings the rainy seasons where you find monsoon rains washing the center of India. These rains start weakening in the month of September, while the rainy season actually ends in the last week of September. The average temperature in the rainy session lies around 36<sup>0</sup> C while the average rainfall is about 35 inches a year (IGFRI, Jhansi, 2013-14 and 2014-15).

Linseed can be raised on different kinds of soils, particularly on silt loam and silty clays. Sandy and badly drained heavy clay soils are, however, unsuitable for the crop. While in central and Peninsular India, it is found to do well on deep clayey black soils, in Indo-Gangetic plains, good crops are obtained on alluvial loams (Sharma *et al.*, 2015).

The soils of Bundelkhand are mainly derived from gneisses. The formation consists of massive rocks traversed by quartz; stand stones, limestones and slates are also found. Bundelkhand soils can be categorized into red soil and black soil (Sharma RB, 2016).

linseed (*Linum usitatissimum* L.) is an important oilseed crop used for industrial and edible purposes. In Turkistan, Afghanistan, India, United States of America, Canada and China, it is primarily grown for seed oil while in Asia and South Russia, it is traditionally cultivated for edible as well as industrial fibre purposes. In Canada, its nutraceutical values are primarily exploited in addition to its fibre for textile industries. linseed plant is endowed with immense qualities. The main industrial use of its oil is in the manufacture of eco friendly paints, varnishes, a wide range of coating oils, linoleum, pad and printing inks, leather and soap. Oil cake is a good feed for milch cattle, which prevents them from many diseases. Recent advances in medicine have established its unparalleled qualities, which controls prostate and bowel cancer and regulates cholesterol. Being a rich source of omega-3 fatty acid, it regulates metabolism of human body and prevents many neurological and brain related elements. Its fibre is lustrous and blends well with cotton, wool and the pulp is used for making currency notes, reinforced plastics and for other artisan purposes (All India Coordinated Research Project on **Linseed, 2018**).

*linicola* is a "diurnal sporulator". In vitro most isolates sporulated only after exposure to diurnal NUV-light. However, for some isolates exposure to diurnal NUV-light did not seem to induce sporulation unless the mycelium was wounded and grown on a medium rich in CaCO<sub>3</sub> (S-medium) at high relative humidity. In vivo sporulation of *A. linicola* was increased after induction by light. The greatest numbers of conidia were produced under continuous leaf wetness and alternating dark/light periods (12 h each). Under these conditions the number of conidia produced increased with increasing temperature from 10°C to 20°C. Alternating 15°C/10°C or 20°C/15°C day/night temperatures decreased the number of conidia produced compared with the constant temperatures 15°C and 20°C, respectively suffers from different physiological and pathological disorder. One of the important pathogen is *Alternaria lini*. It is threat to linseed growers. Different works have carried out investigated work to study the nature of the pathogen.

Few researchers were worked on related field given under following heads:

**Dey, (1933)** first time observed *Alternaria blight* disease on the Government Research Farm of Kanpur and Gorakhpur. He identified it as a new fungus named *Alternaria lini sp.* Mycelium pale olive, spores in chain, dark olive in colour, the second cell form below had the largest diameter, constricted, 3-7 celled measuring 10-40 x 5-10 µ with beak (Av. 24 x 7 µ), beak 3-7 µ (Av. 4.9 µ). In culture Dey could not find any difference in size of conidia. Now since 1933, there were no further reports of any *Alternaria* disease of linseed for another nineteen years till **Arya and Prasada, (1952)** recorded a severe outbreak of a 'blight' disease in the linseed crop in Delhi in the months of March and April (1949). They also isolated an *Alternaria sp.* from the blighted plants that were showing the symptoms somewhat similar to that reported by Dey.

Fungi of *Alternaria nees* genus are widely spread as pathogen on field vegetable, ornamental and orchard plants and cause substantial yield losses in a broad range of host crop species (**Farmers et al., 2004**).

**Groves and Skolko, (1944)** had isolated an *Alternaria sp.* from the seeds of linseed, which they named as *Alternaria linicola* **Johansen, (1943)** earlier reported association of *Alternaria solani* with flax plants. Later on, his material was further examined by **Neergaard, (1945)**, who found it similar to the fungus of **Groves and Skolko, (1944)**.

Leaf spot caused by *Alternaria caucumerina* thrives under humid conditions and is characterized by brown target-like lesion on the leaves. In acute cases, lesions can cover the whole leaf surface and lead to leaf drop. *Alternaria* has caused economic damage in some Queensland guar crops (**Douglas and Routley, 2004**).

Lesions found to appear first on the older leaves as small circular spots. The spots are light brown with a light center and form concentric dark rings as they enlarge thus the name target spot. Lesions which form on the lower leaf surface tend to more diffuse. Fruit infection begins as sunken brown spots and may develop a dark powdery appearance as the fungus sporulates. The infection may also begin at the blossom end with the entire fruit eventually turning brown and shriveling (**Bruce, 2004**).

**Mukhopadhyay et al., (2001)** suggested that flax is increasingly used as an ingredient in feeds for improved animal and fish nutrition. The benefits of omega-3 fatty acids to pigs, cattle, horse and other animals may be in preventing young animals from developing infections.

**Sangwan et al., (2005)** studied the fungal disease of linseed in India viz., rust, Powdery mildew, *Alternaria* blight, wilt, leaf spot, seed rot and seedling blight, including the economic importance, symptoms, causal organisms, epidemiology and control of the disease.

**Siddiqui, (1963)** said that the pathogenic fungus (*Alternaria lini*) was isolated from the affected tissues on Potato Dextrose Agar Medium. The isolated pathogen produced similar symptoms as observed in nature during pathogenicity test.

Small brown, circular oval spots enlarge to 6 cm in diameter. They have definite margins are slightly sunken, often with alternating light and dark zones. Later sporulation on the surface turns the colour to as black (**Walker, 2004**).

## Materials and Methods

### Surface Sterilization of Petri plates and other instruments:

In order to make the experiment free from unwanted microbes, sterilization is prerequisite. For the sterilization of glassware, first the Petri plates and other glassware were thoroughly washed with detergent, water and then sun dried. After the washing, Petri plates were sterilized in the oven at 160 -180°C for 4 - 6 hours.

Many small instruments like forceps, scalpels, needles, bores etc. were ordinarily sterilized by dipping them in 95 % alcohol followed by flaming. These instruments are repeatedly sterilized during the operation to avoid contamination. The mouths of culture vessels were also flamed before pouring or inoculation.

Before inoculation and pouring of the media, hands are repeatedly sterilized with 75 % alcohol to avoid contamination.

### Laminar air flow:

Inoculation of fungus into the Petri plates was done under laminar air flow. Before each experiment, ultra violet radiations were given on for 15 minutes to kill the microbes. After switching of U.V. radiations, the inoculation was done

Collected samples showing typical diseased symptoms were used for isolation of the pathogen. The infected leaves and buds were first thoroughly washed with distilled water.

Cross section of lesion was cut of 5 to 10 mm square, containing both the diseased and healthy looking tissues. Surfaces of the cut portions, were sterilized by dipping in 0.1 % Mercuric chloride (Hg Cl<sub>2</sub>) as surface sterilant solution for 30 seconds. The treated pieces were washed in three washes with sterile water and than dried on clean, sterile paper towels to remove the sterilant. Aseptically transferred the pieces onto sterilized petriplates containing PDA media usually one piece per plate are inoculated. The inoculated plates were incubated in an inverted position at 25 ± 2° C for 3-5 days (Aneja, 1996).

The whitish mycelial growth appeared around the pieces placed in the Petri plates. Further the hyphal tips of mycelium were transferred aseptically in PDA culture tubes the culture obtained from different diseased pieces was subjected to preliminary microscopic examination, which revealed the presence of pathogen responsible for disease development. Finally the culture was purified by single spore technique to keep the fungus viable, active and fresh. Culture of the pathogens was multiplied by regular sub-culturing on PDA both in Petri plates and culture tubes, and was kept in a refrigerator.

After the incubation for 4 days. A temporary mount slide was prepared in cotton blue and lectophenol from various isolates collected from different location and examined under microscope for their shape and size of the pathogen.

## Results

Keeping the objectives of proposed work the experiment was conducted. The *Alternaria lini*, which cause a brown and black target like lesion on the leaves and buds at the binging stage and can cover the whole leaf surface lead to curled and dried of leaves and bud of linseed causing failure of flower to open during the day resulting failure of oval development for seed setting and falling of dried buds after touching by hand or even with speedy wind (Fig 1 &2). Infected capsule did not bear the seeds and at last their capsules were disturbed blighted discoloured and undeveloped.



Figure : 1 Symptoms on Leaves of Linseed Plants

#### Identification of pathogens:

Identification of the isolated pathogen was done on the basis of morphological variations in characters. The characters were same as reported by Dey, (1933). For *Alternaria lini* the morphological characters of the isolated pathogen are given below (Fig 3):

#### Hyphae

The hyphae were Septate, branched, hyaline, colourless turned pale then olive grey. The mycelial width was 3.0 – 5.3  $\mu\text{m}$ .

Conidiophores are septate, erect, branching or non-branching geniculate, olive buff to dark olive buff, 21.5 – 100.5  $\mu\text{m}$  wide.



Figure: 2 Symptoms on buds of Linseed Plants

## Conidia

Conidia are formed singly or in branched chain, consisting 02-10 spores, smooth or verruculose, linear to obclavate, dark olive to buff in colour, provided with 1-7 cross and 0-5 longitudinal septa and often with short conical or cylindrical beak, light brown to dark olive buff, measuring 1.5-45.6 X 7.0-3.5µm in size, beaks usually light colour measuring 3.5-18.0 X 3.0-6.5 µm.

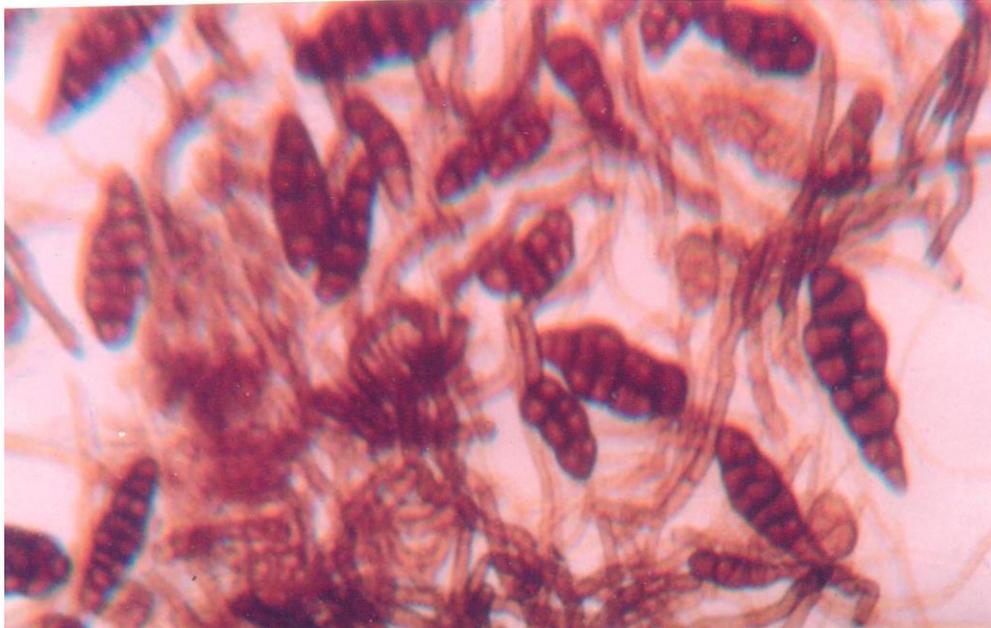


Figure: 3 Conidiophore bearing conidium of *Alternaria lini*

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