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Effect of *Alternaria* on Some Members of Family *Brassicaceae* of Garhwal Himalaya

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Abstract: In the present study a survey of disease caused by genus *Alternaria* on plants of *Brassicae f*amily was carried out and demonstrated the different sp. of *Alternaria* and their causing affect in Brassica family. Four species of Brassica family was taken, witch were infected by different species of pathogens of *Alternaria* family: (1) *Brassica compestris* infected by *Alternaria brassicae* and *A. brassicicola*, (2) *Brassica oleraceae* was infected by *Alternaria brassicae, A. brassicicola* and *A. raphani*, (3) *Brassica nigra* was infected by *Alternaria brassicae, A. brassicicola*, (4) *Raphanus sativus* was infected by *Alternaria brassicae, A. brassicicola, A. rapani*. The best time for the diseased sample collection was January to April. Infected leaf samples were collected in cellophane bag. Samples were collected from the different sites of Srinagar Garhwal and brought to the laboratory for further studies. In laboratory the aseptic conditions was maintained to isolate the pathogen on potato dextrose agar media and as well as in blotter papers. After incubation shape and size of conidiospores of different spp. of *Alternaria* were studied. [New York Science Journal. 2009;2(6):80-85]. (ISSN: 1554-0200).

Introduction:

Brassicaceae plays an important role for its uses as vegetable as well as oil yielding seed. Most of the plants of the family contain plenty of sulphur compounds. These crops are cultivated in farm in leafy form and/or to produce in seed form. It is a large family contains 350 genera and about 2500 species. This family is cultivated in whole India, but in specially Garhwal region, peoples are cultivating *Brassica compestris, Brassica nigra, Brassica oleraceae L, Brassica juncea, Raphanus sativus L, Iberis amara* in large area.

The leaf spot & seed disease caused by various species of *Alternaria* occur abundantly in some region of Garhwal Himalayas. This disease is so prevalent that most of the plants of the area were found to be infected by this disease. The disease of then become so severe that growth & yield of the crop decreased considerably. Species of *Alternaria* which is a member of Deuteromycetes are facultative parasite causing disease on most of the common and economical important crop plant. It is very common fungi often found on plant debris and on living plant parts. It produces very distinct symptoms on foliar parts of the plant (Agarawal and Hasija, 1967). The fungal genus *Alternaria* is comprised of many saprophytic and endophytic species, but is most well known as containing many notoriously destructive plant pathogens. There are over 4,000 *Alternaria*/host associations recorded in the USDA Fungal Host Index ranking the genus 10th among nearly 2,000 fungal genera based on the total number of host records. While few *Alternaria* species appear to have a sexual stage to their life cycles, the majority lack sexuality altogether. Many pathogenic species of *Alternaria* are prolific toxin producers, which facilitates their necrotrophic lifestyle (Christopher, 2008). *Alternaria* produces toxin(s) which are responsible for these lesions. Understanding the mechanism of action, particularly, host specific toxins (HSTs) provide a better appreciation of host pathogen interactions and resistance mechanisms. Two approaches have been used to study the mode of action of HSTs. One is the study on the molecular level of host selectivity and the other at the cellular level. It causes amongst other aberrations in chloroplast and mitochondria. It cause sever infection on most of the family *Brassicaceae* viz. *Brassica nigra, Bressica campestris Linn, Brassica oleraceae, Raphanus sativus, Iberis amara* as a result of which the quality and quantity of crop decreases (Johnson et al., 2000).

Many unanswered questions regarding fungal pathogenicity, especially pertaining to *Alternaria* species, still remain. All of the plant pathogenic *Alternaria* species to date have been reported to produce host-specific toxins (HSTs) and/or nonhost specific toxic substances, both having very diverse biochemical structures (Rotem**,** 1994; Thomma, 2003).They affect the quantity as well as quality also. Thus it is necessary to study the details about the occurrence, symptomology and morphology of the pathogen.

Keeping above points on view the objectives of the present investigation was to study the symptoms, disease development, morphology and identification of the pathogen on various hosts.

Material & Methods:
The details of the material and methods used during the present investigation are as follows:

Collection of Plant Samples:

Infected samples of *Brassicaceae* were collected from Srinagar Garhwal and its adjoining areas. These localities were: Srinagar, Government nursery, Dang, Bhaktiyana, Shaktivihar, and Ghasiamahadev. The sites of collection include cultivated fields, kitchen garden, road sides etc. After collection of samples, these were carried out to the laboratory and isolated within two or three days after collection.

Isolation of Alternaria:

In order to study the disease of *Alternaria* in *Brassicacea*, the following experiments were performed by two methods:

The blotter test:

The blotter test is a combination of the *in vitro* and the *in vivo* principles of investigation. The basic principle in this method was to provide a high level of relative humidity, and optimum light and temperature those are conductive for fungal development. Usually three layers of blotter papers were provided enough moisture for duration of the test. Blotters were soaked in tape water and placed in culture plates after draining off the excess water. The thickness of filter paper in Petri dishes is having depth of 2.0 cm. The leaves samples were placed in to the Petri dishes. The samples were incubated for a fixed period of time, usually one week, at the fixed temperature that is 20± 2^0c. The fluorescent light used with a 12 hr. cycle of light and dark. The essence of recording in blotter test is quick identification of habit characters. *Alternaria* species displaying characteristic features such as the form, length and arrangement of conidiophores; the form size, septation, colour, chain formation, etc., of conidia and their arrangement on the conidiophores.

Incubation of samples in Petri dishes containing tap water agar. *(Agar, 15 gm; tap water 100 ml)**:***

Small pieces of samples were placed in each dish. Prior to plating, samples were treated with 1-2% sodium hypo chloride (NaOCl) solution to prevent saprophyte development. Malt extract agar or potato dextrose agar media most commonly applied. The samples were incubated at a fixed temperature, mostly about room temperature 20±2^0c for an incubation period of 5 to 8 days for the development of fungi. The principle of recording in the agar plate test is macroscopically examination of fungus colonies. Isolation of Alternaria species was made by direct transfer to suitable media by means of sterile inoculating needles or fine forceps. Potato dextrose (peeled potatoes 200gm; dextrose20gm; agar 20gm and water 100ml) (Ricker and Ricker 1936) was routinely used for isolation of such fungi.

Identification of Alternaria Species:

An attempt was made to identify all species of *Alternaria* which appear on infected plant parts. For identification, monograph of *Alternaria* was consulted. Microscopic observation was carried out using living specimens mounted and water and lactophenol cotton blue.

Results and Discussion:
***Alternaria brassicicola* on *Brassica oleraceae* (black leaf spots disease):**

The pathogen known to cause the most destructive and widespread *Alternaria* leaf spots, speckle disease, dark leaf spot or pod spots. This disease is attack mostly on *Brassica oleracae*. Infection of *Alternaria brasicicola* in seed resulted discoloration, shriveling and changes in seeds contents (Schimmer, 1953). Weakly discoloured and symptomless seeds revealed the mycelial fragments in outer seed coat layers. An early study in cabbage revealed Alternaria brassicicola restricted to seed coat only and spores on seed surface. Heavy aggregation of mycelium and in the vicinity of hilum suggests entry of pathogen through hilum. These confirm that mycelium was found aggregated in the cavities and folds of the tissue in the hilum region. The cells of palisade layer of seed coat showed reduced thickenings mycelial fragments.

This indicates that mycelium can also penetrate directly through the seed coat layer (Chahal *et al.*, 1979; Tonukari, 2000).

A brassicicola germinate *(in vitro)* at a higher temperature (tested at a temperature range of 7 to 31^0C). *A. brassicicola* begins to germinate 98% of its spores at 15^0C after 10 hrs. of incubation. Plants wound inoculated with *A. brassicicola* develop symptoms most quickly at 25^0C, while seedlings from infected seed develop symptoms most quickly at 30^0C. Free water or high humidity is required for germination and infection. Germination also requires the presence of moisture in the form of free water or high relative humidity (at least 95%). Seeds infected with *A. brassicicola* are known to have active surface spores for up to 2 years when the seeds are stored at 10^0C with 50% relative humidity. Internal mycelium can remain viable for upto 12 years. *A. brassicicola* also survive in the form of microsclerotia and chlamydospores which appear after infected leaves have partially decayed. Microsclerotia and chlamydospores of both pathogens can be formed within conidial cells. Both microsclerotia and chlamydospores develop best at low temperature (3^0C) and are resistant to freezing and dessication (in vitro). Chlamydospores also can develop in conidial cells on natural soil at room temperature (Neergaard, 1945).

The symptoms were appeared as dark colored necrotic spots on the leaves, spreading rapidly and forming circular concentric rings/lesions up to one cm in diameter, black sporulation was also seen on the spot (Plate-1). Conidiophores amphigenous arising singly or in group of 2-13 or more through the stomata 0-4 septate with the basal cell sometimes slightly swollen olivaceous merely branched straight and upright when solitary, often curled when fasiculate not markedly geniculate usually with a single terminal scar sometimes with lateral spores also 20 – 60 x 4 – 9 µm. Conidia born in chain of up to 20 or more. The conidia spread to siliqua , the germ tube penetrate through the pericarp in to the seed coat of maturing seed and hyphae establish as dormant mycelium (Riker *et al.*, 1936).

***Alternaria alternate on Brassica campestris* (Leaf spot disease):**

It causes leaf spot on rape and mustard and has been found to be associated with rape. In rape the dark thick knotty mycelium was limited to seed coat layers in bold symptom less and weakly discolored seeds. Heavy inoculum at hilum reason and weakened radical thickening of palisade layer in seed coat suggested mode of penetration and ways similar to *Alternaria brassicicola* (Jain *et al.*, 1970).

The disease makes its appearance brown nacrotic spots on cotyledonary leaves and brown streaks on hypocotyl in seedlings leading to post emergence losses. At maturity, it naturally infected and inoculated plants, it cause dark brown necrotic spot on stem and ashy brown oval to irregular spots on pods. In leaves the spot becomes circular to irregular and shape with dirty grey center and dark brown margins and sometimes coalesce to form irregular patches. Germinated conidia of pathogen secrete cytokinins which lead to the formation of Green Island below the germinating conidia on senescing tissues (Chaturvedi, 1972; Zheng, 2006).

***Alternaria raphani* on *Brassica nigra* (Leaf spot disease)**

The initially appear as small scattered grey spot on leaf lamina. Later these, spread rapidly to form almost circular spot 3-8 mm in diameter, brown to dark with raised yellow margins and distinct zonations. Formation of chlamydospore on hypocotyle, stem and seeds with finally get killed. Necrotic spot on cotyledonary leaves and hypocotyls were also observed. A. raphani chlamydospore are considerd important for overwintering in nature and also the longer survival up to 15 years of the fungus in soil culture is attribute to formation of chlamydospore (Kothari *et al.*, 1970).

The pathogen may survive as dormant mycelium or spores on seeds as it has been reported on rape and mustard plants.After the study of mycelium it is observed that the mycelium is septate, branched, whitish to greenish, gray, aging to dark olive 3.5-7.5µm in width.Conidiophores are simple cylindrical erect or somewhat curved septate (3-7), grayish olive and 3.0-6.5µm in size. Conidia produced singly or in short chain of 2-3 are irregular oval, light grayish olive to grayish olive and smooth with 3-10, constricted at septa. The conidia measures 20-60*11.5-26 µm in size (Thomma, 2000).

Epidemiology:

Infected seeds, with spores on the seed coat or mycelium under the seed coat, are likely the main source of transport of these pathogens. Spores are disseminated by wind, water, tools and animals. The fungus can survive in susceptible weed or perennial crops. Infected crops left on the ground after harvest

also serve as a source of infection for *Alternaria brassicae* and *A. brassicicola*. In one study, Infected leaves of oilseed rap and cabbage placed out doors on soil, produced viable spores for as long as leaf tissues remained intact. For oilseed rape this was up to 8 weeks and for cabbage up to 12 weeks. This type of spread is likely to occur in seedling beds as well, and seedling from infected seed beds can carry the inoculum to the field (Sreekanthian *et al.*, 1973).

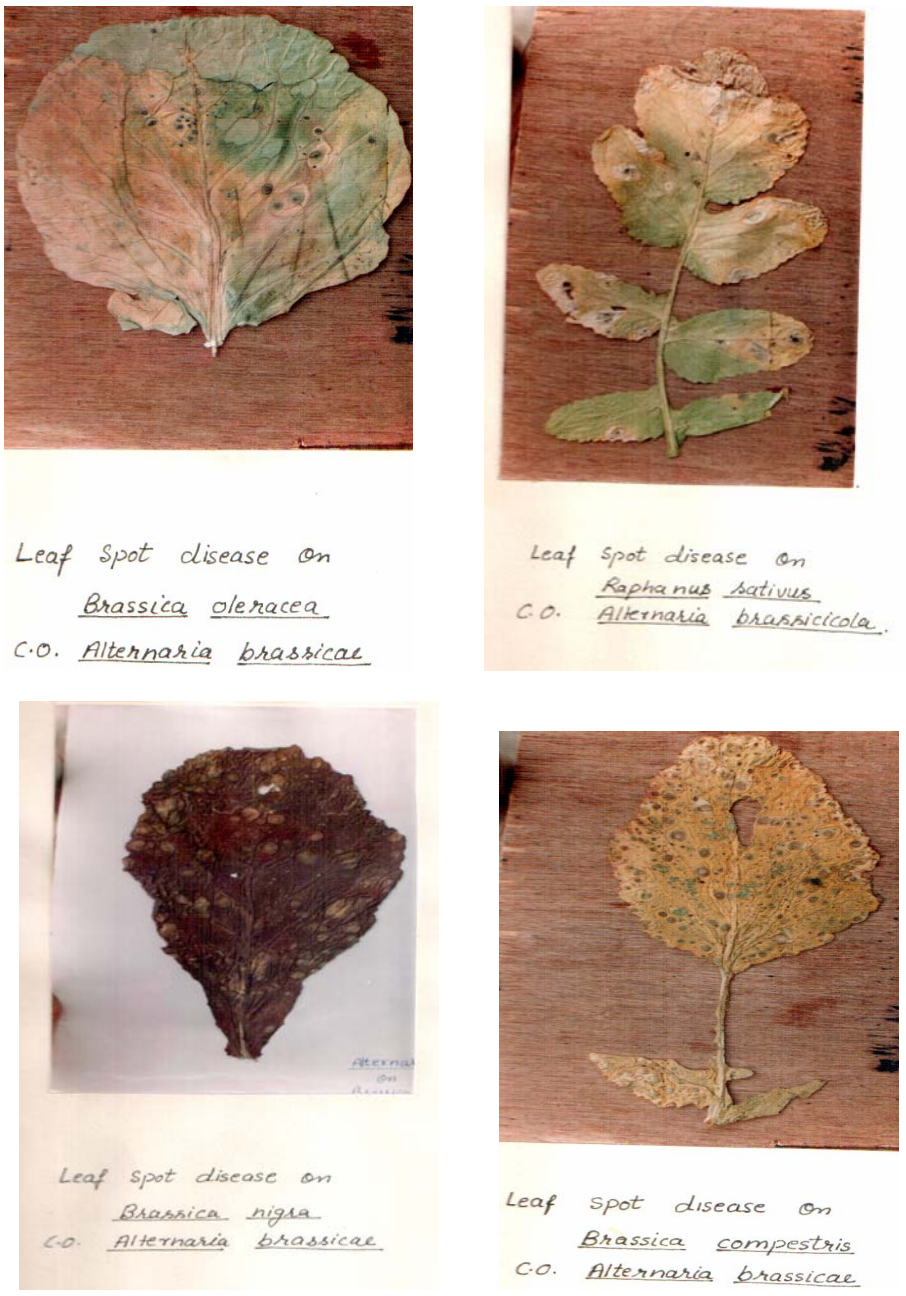

Plate-1 Leaf Spot Diseases on different Species of *Brassica*

Symptomology:

After disease attack of *Altenaria* there occurs several symptoms on the plant of Brasicca family. The physiological changes occur in the plant after infection of Alternaria. There occur several anatomical

and morphological changes which are expressed externally in form of visible symptoms. Wadhwani and Dudeja (1982) have described the development of the disease in three phases.

1. On leaves in contact with soil when relative humidity was high.
2. After pollination (early fruit set stage) when there were heavy rains.
3. On fruit.

When the host plant has passed the seedling stage, infections generally become confined to superficial, scarcely visible necroses, often located at the soil level. Later infections may spread to weakened parts of the host such as dying leaves and petals or any has tissues losing vitality as they do during the maturation of seed. These conditions give an opportunity for the pathogen to get a foothold in the developing seeds, thus determining the rate of seed infection. The fungus of *Alternaria brassicae, A. brassicicola* may penetrate the pod at different times during its development and directly invade the maturing seeds. *Alternaria brassicae* first appear on the cotyledonary leaves as small light brown spots, later turning black due to sporulation of the pathogen and as necrotic streaks of hypocotyls. On leaves, the infection starts as brown to blackish point which enlarges becoming entirely black or dark bordered with a gray centre. The spots spread rapidly on the stem and pods and become more or less circular (0.5 to 10 mm in diameter), slightly raised above leaf surface. Linear spots have also been reported on stem. In severely infected plants, the stem is so heavily attacked that it undergoes defoliation even before the pods reach maturity. The pathogen penetrates into the pods and infects the seeds which may show gray to brown discoloration. *Alternaria brassicicola* caused dark coloured necrotic spots on leaves spreading rapidly and forming circular concentric rings/lesions upto one cm in diameter. In humid weather the fungus may produce bluish tinge in the centre of these spots. Dark discoloration at the base of hypocotyl, sometimes extending on stem as streaks and dark spots on cotyledons were observed. The pathogen harboured as dormant mycelium in the seed coat or as condial contamination is carried on the cotyledons and in the seed coat and transmitted to young plants by air currents and/or rain splashes (Kaul and Narain, 1981.).

Scarcely visible necrotic symptoms on the stem at the ground level or in weakened leaves are often located. *Altenaria raphani* shows formation of chlamydospores on hypocotyls, stem and seeds which finally get killed. Nectotic spots on cotyledonary leaves and hypocotyls were also observed. The chlamydospores of A. raphani are considered important for overwintering in nature and also the longer survival upto 15 years of the fungus in soil cultures is attributed to formation of chlamydospores. The pathogen may survive as dormant mycelium or spores on seeds.

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