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Histopathological studies of *Alternaria alternata* associated with carrot seed, *Daucus carota* L.

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Abstract
Carrot is an important root vegetable crop of Rajasthan. The crop suffers severely from various seed borne diseases. *Alternaria* leaf blight is an important disease of carrot. All seed components viz. pericarp, endosperm and embryo were employed by two methods viz., component planting and whole mount method. Maximum (4-56 and 3-43%) percent infection of *A. alternata* was observed in pericarp followed by endosperm (2-42 and 1-39%) and it was absent in embryo (0 and 0%) in both the methods.

Keywords: carrot, *Alternaria alternata***,** pericarp, endosperm, and embryo

1. Introduction
The carrot (*Daucus carota* L.) belongs to family: Umbelliferae, is a root vegetable, usually orange in colour. Carrot roots are used as a vegetable for soups, stews, curries and pies; grated roots are used as salad, tender roots as pickles (Bose *et. al*., 1986) [2]. One of the important factors which limit the production of this crop in Rajasthan is the use of contaminated seeds and following of traditional package of practices for the cultivation of carrot by farmers culminating into heavy losses at all the stages of crop growth till harvest. The crop suffers from several numbers of phytopathogenic fungi. In most of the carrot producing areas, *Alternaria* leaf blight (ALB) is recognized as the most common and destructive foliage disease in carrot (Clerc *et al.*, 2009 [4] and Boedo *et. al*., 2010) [1]. In Israel and Turkey, the disease incidence was recorded 65 to 90% with reduced root yields about 40-60% (Noon *et al.*, 2001; Netzer and Kenneth, 1969 and Vintal *et al.*, 1999) [9, 8, 13] that decreased the effectiveness of mechanical harvesting (Bragg lumber 1999; Noon *et al.*, 2001 and Soylu *et al*., 2004) [3, 9, 12]. Initial symptoms of the disease is first appeared on older leaves as irregularly shaped, minute, dark brown to black spots with yellow borders on the edge of the leaflet blade. As the disease progressed the lesions expanded the leaflets to turn brown, shrivel and die.
The experiment was conduct to observe the exact location of *Alternaria alternata* in the seeds it has become essential to carry out an experiment on its detection. The location of pathogen in seed may be of relevance to its transmission and further pathogenesis.

2. Materials and Methods
To observe the exact location of *Alternaria alternata* in the seeds collected, two methods Component Plating and Whole Mount (Singh *et al.*, 1980) [10] were followed.

2.1 Component Plating Method: The method suggested by Singh *et al.* (1980) [10] was followed with slight modification. Fifty seeds from highly contaminated sample (A) were selected at random and used for detection of fungi. Seeds were thoroughly washed (one seed per test tube) three times with tap water and finally with sterilized distilled water and then soaked in distilled water for 7-8 hours. Each seed was then dissected aseptically in to different parts i.e. pericarp (seed coat), endosperm (cotyledon) and embryo (embryal axis) with a pair of sterilized needles under stereo-bionocular microscope. Each component was surface disinfected with 0.1 per cent $HgCl_2$ solution followed by three washing with sterilized distilled water and then components of individual seed were plated at equal distance in Petri dish having three moistened blotting papers. The dishes were incubated at 24$\pm$ 1 ^{0}C under 12 hour's alternative cycles of light and darkness. After 7 days, seed components were examined for presence of *Alternaria alternat*a.

2.2 Whole Mount Method: One hundred seeds of highly contaminated sample (A) were taken at random and used for detection. Seeds were boiled individually in distilled water for 5-7 minutes and allowed to cool.

Each seed was dissected aseptically to separate the seed components (i.e., pericarp, endosperm and embryo) with a pair of sterilized needles under stereo-binocular microscope. These parts of seeds were boiled individually in 10% HCl for 10 minutes in test tubes. After cooling they were washed thoroughly with tap water and finally with sterilized water. Tissues of components of seed were macerated on a slide followed by staining and mounting in cotton blue and lactophenol, respectively. The slide was examined under compound microscope and per cent infection of percarp, endosperm and embryo by fungi was recorded method suggested by Jha, 1995 [5].

3 Result and Discussion

3.1 Component plating method: *Alternaria alternata* were observed only in pericarp and endosperm (Table 1 and Fig. 1). Incidence of this fungus was higher in pericarp than endosperm but it was absent in embryo. The maximum per cent infection of *Alternaria alternata* was observed in pericarp and endosperm from sample "A" (56.00 and 42.00 %) followed by 'C' (48.00 and 37.00 %), 'B' (32.00 and 20.00 %), 'D' (19.00 and 10.00%) and it was minimum in sample "E" (4.00 and 2.00 %), respectively.

3.2 Whole mount method

Microscopic examination of mounted components of seed revealed the presence of hyphae and conidia of *Alternaria alternata* in pericarp and endosperm (Table 2 and Fig.2) but not from embryo. The per cent infection of *Alternaria alternata* in pericarp and endosperm were observed maximum in sample "A" (43.00 and 39.00 %) followed by 'C' (41.00 and 32.00 %), 'B' (29.00 and 20.00 %), 'D' (15.00 and 8.00 %) and minimum in sample "E" (3.00 and 1.00 %), respectively.

The location of pathogen in seed may be of relevance to its transmission and further pathogenesis. The histopathological study of *A. alternata* in all seed components viz. pericarp, endosperm and embryo were employed by component planting and whole mount method. Incidence of *A. alternata* observed to be more in pericarp in comparison to endosperm but not from embryo. Similar results were achived by Netzer and Kenneth, (1969) [8]; Neergaard, (1977) [7]; Soteros, (1979) [11] and Kim and Mathur, (2006) [6].

Table 1: Detection of *A. alternata* in parts of carrot seed by component plating method

Samples	Per cent component showing infection*		
	Pericarp	Endosperm	Embryo
A	56	42	0
B	32	20	0
C	48	37	0
D	19	10	0
E	4	2	0

*No of seeds tested = 50

A = Kelanwas, B = Nimbi, C = Surethi, D = Todameena, E = Company produce

Table 2: Detection of *A. alternata* in parts of carrot seed by whole mount method

Samples	Per cent component showing infection*		
	Pericarp	Endosperm	Embryo
A	43	39	0
B	29	20	0
C	41	32	0
D	15	8	0
E	3	1	0

*No of seeds tested = 100

A = Kelanwas, B = Nimbi, C = Surethi, D = Todameena, E = Company produce

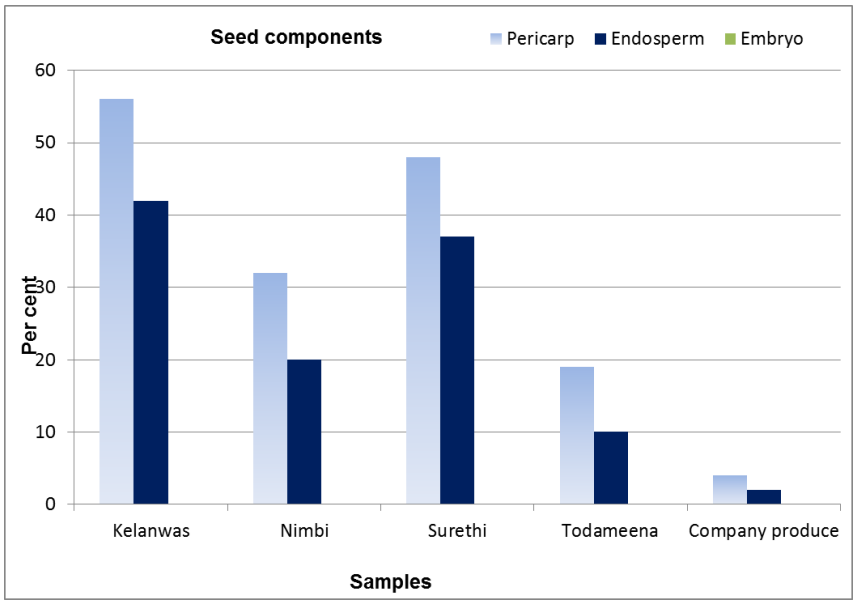

Fig 1: Detection of *A. alternata* in parts of carrot seed by component plating method

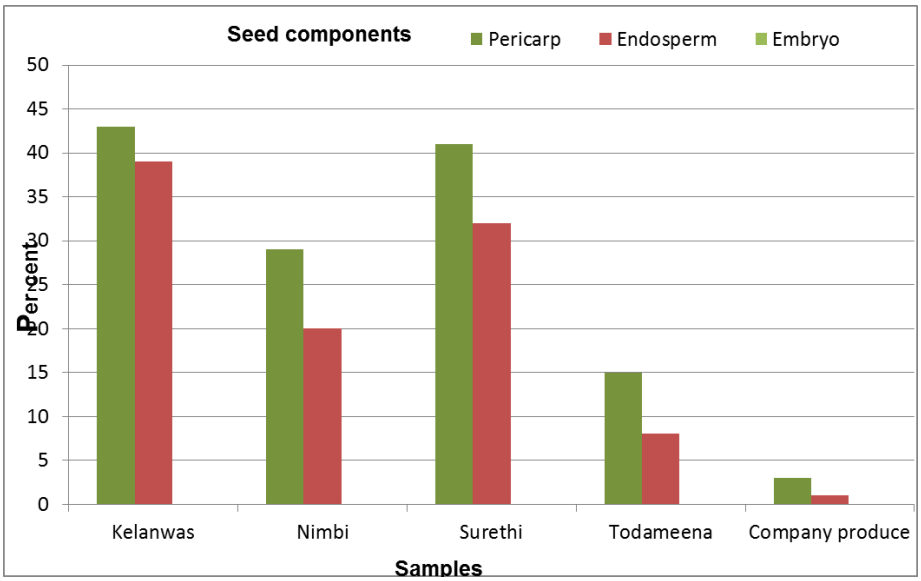

Fig 2: Detection of *A. alternata* in parts of carrot seed by whole mount method

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