

INCIDENCE AND HISTOPATHOLOGY OF *ALTERNARIA ALTERNATA* IN SUNFLOWER SEEDS

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One hundred eighty eight seed samples of sunflower (*Helianthus annuus L.*) revealed asymptomatic and symptomatic (bold-discoloured and shrivelled-discoloured) seeds. Seeds with greyish brown and brown to black discolourations yielded *Alternaria alternata*. The fungus was recorded in 133 seed samples with an incidence range of 1-98%. The pathogen was located in pericarp of asymptomatic seeds whereas, in pericarp, endosperm and embryo of bold-discoloured and shrivelled-discoloured seeds. The mycelium was inter- and intracellular. Seeds with severe infection showed a heavy aggregation of mycelium in the inner layers of the pericarp, space between endosperm and embryo and cells of cotyledons and shoot apex.

Keywords: *Alternaria alternata*; Histopathology; Incidence; Seeds, Sunflower.

Introduction

The frequent occurrence of *Alternaria alternata*, internally in cereal seeds is well documented¹⁻³. In oilseeds it has been recorded in linseed⁴, rape and mustard^{5,6} and taramira⁷. *A. alternata* causes leaf spot in sunflower⁸⁻¹⁰. During routine seed health testing of samples collected from Rajasthan, it was found to occur in large number of seed samples of sunflower; therefore, a study of its incidence in seed lots and location in seeds was made.

Materials and Methods

One hundred eighty eight seed samples collected from 11 districts of Rajasthan during 1992-94 were tested by dry seed examination and blotter test¹¹ to study the incidence of *Alternaria alternata*. Location of *A. alternata* was studied in two naturally infected seed samples (ac. nos. 8504 and 8512) carrying 85% and 89% incidence respectively, using methods of component plating, clearing and wholemount preparations and microtome sectioning¹².

Results and Discussion

Incidence in seed samples : Seed samples of sunflower on dry seed examination revealed

bold-symptomless, bold-discoloured and shrivelled-discoloured type seeds (Fig.1). Seeds with greyish-brown and brown to black discolourations on incubation yielded growth of *Alternaria alternata*. In blotter test, 43 fungi of 23 genera were isolated from seeds of which *A. alternata* was recorded in 133 seed samples with incidence range of 1-95% in untreated seeds and 1-98% in pretreated seeds. Samples from Jhalawar, Jaipur, Kota, Baran and Bhilwara revealed relatively high infection percentages.

The heavy infestation of seed samples (70.74%) with *A. alternata* (1-98%) from 11 districts suggests its widespread occurrence in Rajasthan. By producing discolourations on surface it affects the seed quality adversely. Similar observations have also been reported in rape and mustard^{5,6}, coriander¹³ and sunflower¹⁴.

Histopathology of *A. alternata* infected seeds: In component plating, growth of *A. alternata* (Fig.2) was recorded on outer- and inner pericarps in bold-symptomless seeds whereas in outer- and inner pericarps, endosperm and embryo in bold-discoloured and

Table 1. Percentage infection of *A. alternata* in different parts of seeds of sunflower in component plating and cleared wholemount preparations.

SEED COMPONENTS	SEED CATEGORIES					
	Ac.no.8504			Ac.no.8512		
	BS	BD	SD	BS	BD	SD
I. COMPONENT PLATING:						
Seed coat						
Outer pericarp	22	70	100	10	62	100
Inner pericarp	12	66	100	10	52	100
Endosperm	00	50	78	00	40	74
Embryo						
Cotyledons	00	36	60	00	28	40
Embryal axis	00	16	24	00	16	26
II. CLEARED AND WHOLEMOUNT PREPARATIONS :						
Seed coat						
Outer pericarp	14	60	100	10	40	100
Inner pericarp	10	56	100	10	38	100
Endosperm	00	36	68	00	24	72
Embryo						
Cotyledons	00	20	30	00	18	28
Embryal axis	00	10	18	00	10	18

BS = Bold-symptomless; BD = Bold-discoloured; SD = Shrivelled-discoloured

shrivelled-discoloured seeds in both the samples (Table 1).

Cleared wholemount preparations revealed thick, dark, branched and septate mycelium of *A. alternata* in outer pericarp (Fig.3) and inner pericarp in bold-symptomless seeds whereas in outer and inner (Fig.4) pericarps, endosperm layer (Fig.5) and embryo (Fig. 6) of bold-discoloured and shrivelled-discoloured seeds in both the samples (Table 1). Conidia of *A. alternata* were also observed in seed coat of some seeds.

Microtome sections showed mycelium of the pathogen in outer and inner pericarps of all the seed categories (Fig.7 & 11). In 3 out of 10 bold-discoloured seeds, mycelium penetrated the cuticle and formed small compact knots or cushions in the inner pericarp more abundantly at the hilar end. The cuticle

was relatively thin and sinuate with gaps at places. Abundant mycelium was observed in space between endosperm and embryo, between the cotyledons and cells of cotyledons and shoot apex (Fig. 8-10) in severely infected seeds. Thus, *A. alternata* in sunflower seeds was found to be extra as well as intraembryal in nature. Similar observations were also made by Singh *et al.*⁸, Raut¹⁵, Kaur *et al.*¹⁶ and Krishnappa and Shetty.¹⁷ Its mycelium in seed coat, endosperm and embryo in infected chilli seeds has also been reported from Rajasthan. In shrivelled- discoloured seeds the parenchyma, aleurone and endosperm layers were completely disintegrated and replaced by mycelium. The embryo was greatly reduced and the cells showed complete depletion of contents.

The presence of mycelium of *A. alternata* in seeds of sunflower in outer- and

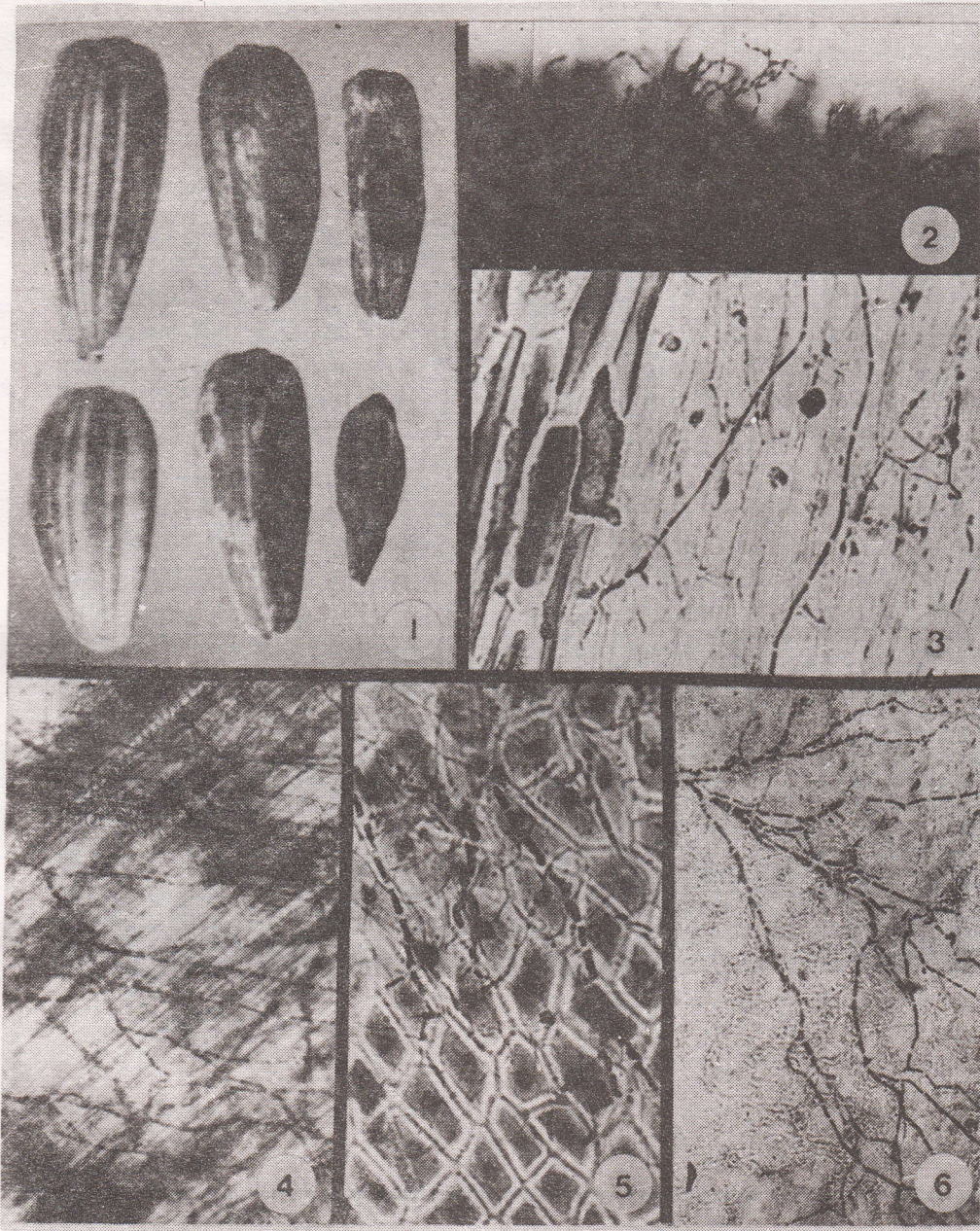


Fig. 1-6: Infection of *Alternaria alternata* in sunflower seeds; Fig.1. Seed Categories (left to right) bold-symptomless, bold-discoloured and shrivelled-discoloured seeds. Note greyish brown and brown to black discolouration on seed of later two categories. x 20; Fig. 2. Chain of conidia of fungus on seed surface, x 50; Figs. 3-6. Seed components showing thick, dark, septate, inter- and intra cellular mycelium in cleared wholemount preparations. (3) outer pericarp (4) inner pericarp (5) endosperm and (6) cotyledon. x 125.

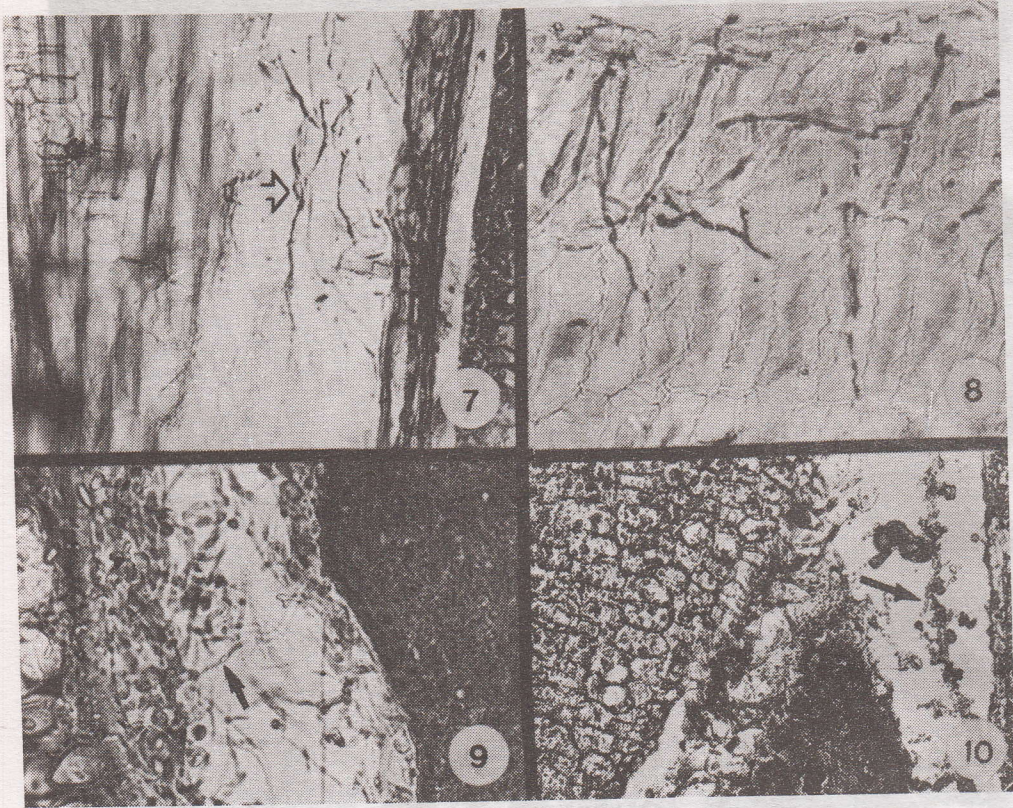


Fig. 7-10: Histopathology of infected seeds; Fig. 7. mycelium in seed coat layers. x 25; Fig.8. inter - and intra cellular mycelium in the cotyledonary tissue. x125; Fig. 9. abundant mycelium in space between endosperm and embryo. x 125; Fig. 10. heavy colonization of shoot apex and space between the cotyledons. x 125.

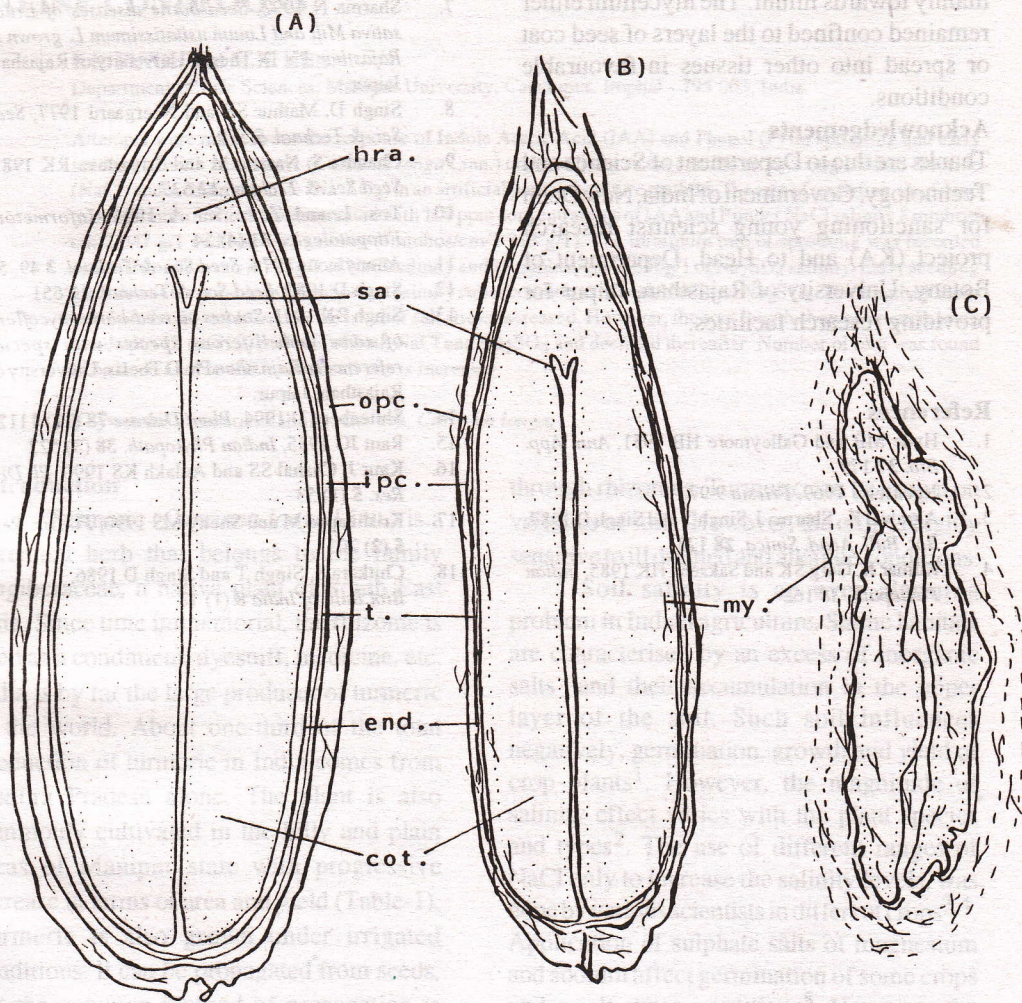


Fig. 11: Semidiagrammatic representation of section of categorised seeds showing expanse of mycelium of *Alternaria alternata* in sunflower.

- A. Bold- symptomless seed
- B. Bold- discoloured seed
- C. Shrivelled- discoloured seed

(Cot. - Cotyledon; end. - endosperm; hra - hypocotyledonary root axis; ipc - inner pericarp; my- mycelium; opc - outer pericarp; sa - shoot apex; t - testa)

inner pericarp layers of seed coat in bold-symptomless seeds suggests that the pathogen penetrate seed through epidermis mainly towards hilum. The mycelium either remained confined to the layers of seed coat or spread into other tissues in favourable conditions.

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