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HISTOPATHOLOGY OF *ALTERNARIA BRASSICICOLA* INFECTED MUSTARD SEEDS

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Three seed samples of mustard (*Brassica juncea* Coss.) with 52%, 60% and 63% natural infection of *Alternaria brassicicola* respectively were studied. Seeds were categorized into bold-symptomless, bold-discoloured and shrivelled-discoloured types. The inter - and intracellular mycelium of *A. brassicicola* was confined to the seed coat in bold-symptomless and bold-midly discoloured seeds and occurred in all the parts of bold-discoloured and shrivelled-discoloured seeds. The infected cells showed depletion of contents and necrosis. The infection of *A. brassicicola* in mustard seed is extra- as well as intraembryal.

Key words: *Alternaria brassicicola*, seed-borne, histopathology, mustard.

Alternaria brassicicola (Schw.) Wilts is a serious seed-borne pathogen of crucifers causing leaf spot disease. The seed-borne infection acts as source of primary inoculum (Neergaard, 1977). A study on seed-borne mycoflora has revealed the occurrence of *A. brassicicola* in 50% seed samples with 0.5-100% incidence (Sharma, 1989). The present study presents a detailed account of location and histopathology of the pathogen in naturally infected mustard seeds.

MATERIALS AND METHODS

Three seed samples of mustard cultivars T-59 (accession nos. 2573 and 2810) and RL-18 (ac. no. 2929) which contained 60,63 and 52 per cent natural infection of *Alternaria brassicicola* (Fig. 2) as revealed by blotter test were used for the study. The procedures of component plating, clearing and wholemount preparations (50 seeds/category per sample) and microtome sectioning (10 seeds/category per sample) as described by Agrawal *et al.* (1987) were followed.

RESULTS

On the basis of dry seed inspection; the seeds were categorized into three categories namely (i) bold-symptomless, (ii) bold-discoloured and (iii) shrivelled-discoloured seeds (Fig. 1). The results of histopathological studies in various seed categories are described below.

Component plating : The seed coat alongwith endosperm (as the two compcnents could not be seperated) and embryo were incubated. Growth and sporulation of *A. brasscicola* were observed on seed coat (alongwith endosperm) of 42, 38 and 26 per cent bold-symptomless seeds (category I) in sample nos. 2573, 2810 and 2929 respectively, whereas it occurred on 100% seed coat of bold-discoloured (category II) and shrivelled-discoloured (category III) seeds of all the samples. No growth of the pathogen was observed on embryo in seeds of category I. In III category, embryo in 22, 18 and 42 per cent seeds and in III category 42, 24 and 60 per cent seeds revealed the growth of the pathogen in three samples respectively.

Clearing and wholemount preparations: Thick, septate, branched, inter-and intracelular mycelium of *A. brassicicola* was observed in seed coat epidermis of 48, 38, and 32 per cent bold-symptomless seeds in sample nos. 2573, 2810 and 2929 respectively but it could not be located in endosperm and embryo. Besides mycelium, spores of *A. brassicicola* were also recorded in seed coat epidermis (Figs. 3 and 4). In seeds of II and III categories, the hyphae frequently occurred in epidermis and palisade layer of seed coat, endosperm (Fig. 5) and embryo (Fig. 6). In embryo, it occurred in 20, 14 and 22% in II category seeds and 36, 24 and 54% in III category seeds in the three samples respectively.

Microtome sections : Sections of seeds of different categories revealed the precise distribution of fungal hyphae in various seed compcnents. Out of 10 bold-symptomless seeds examined from each sample, 5, 4 and 2 seeds in sample nos. 2573, 2810 and 2929 respectively had the mycelium of *A. brassicicola* confined to the seed coat epidermis. The mycelium was

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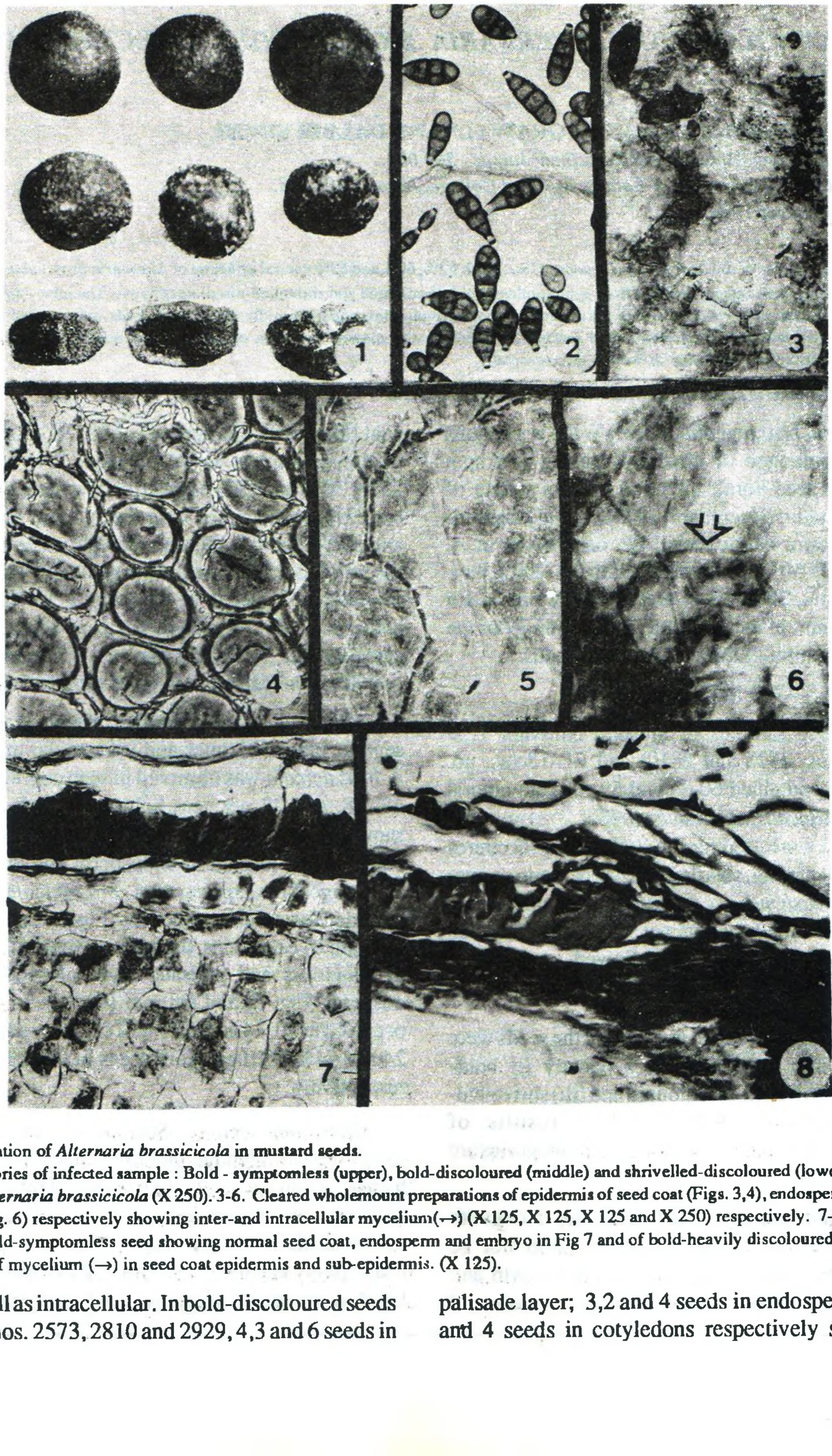
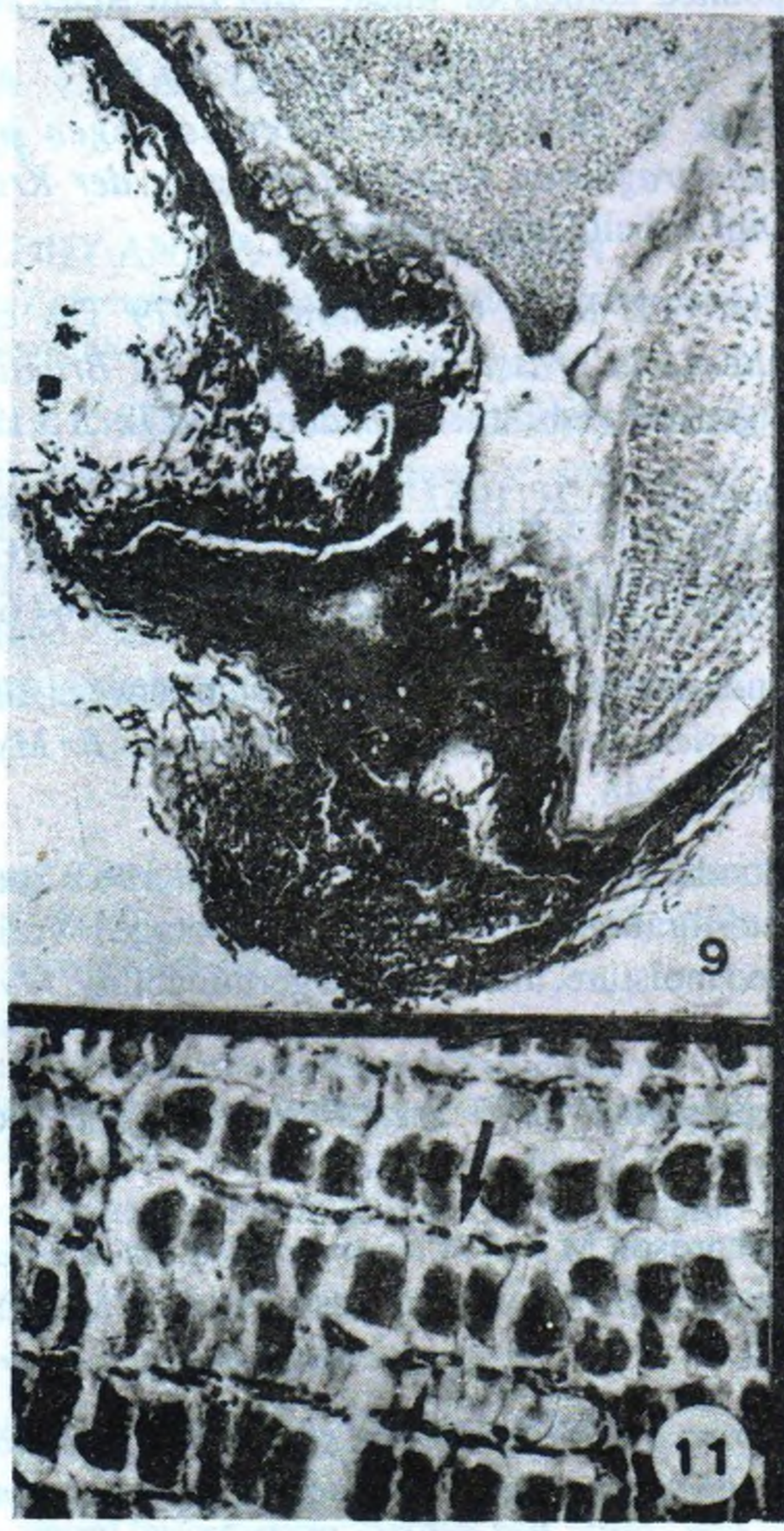
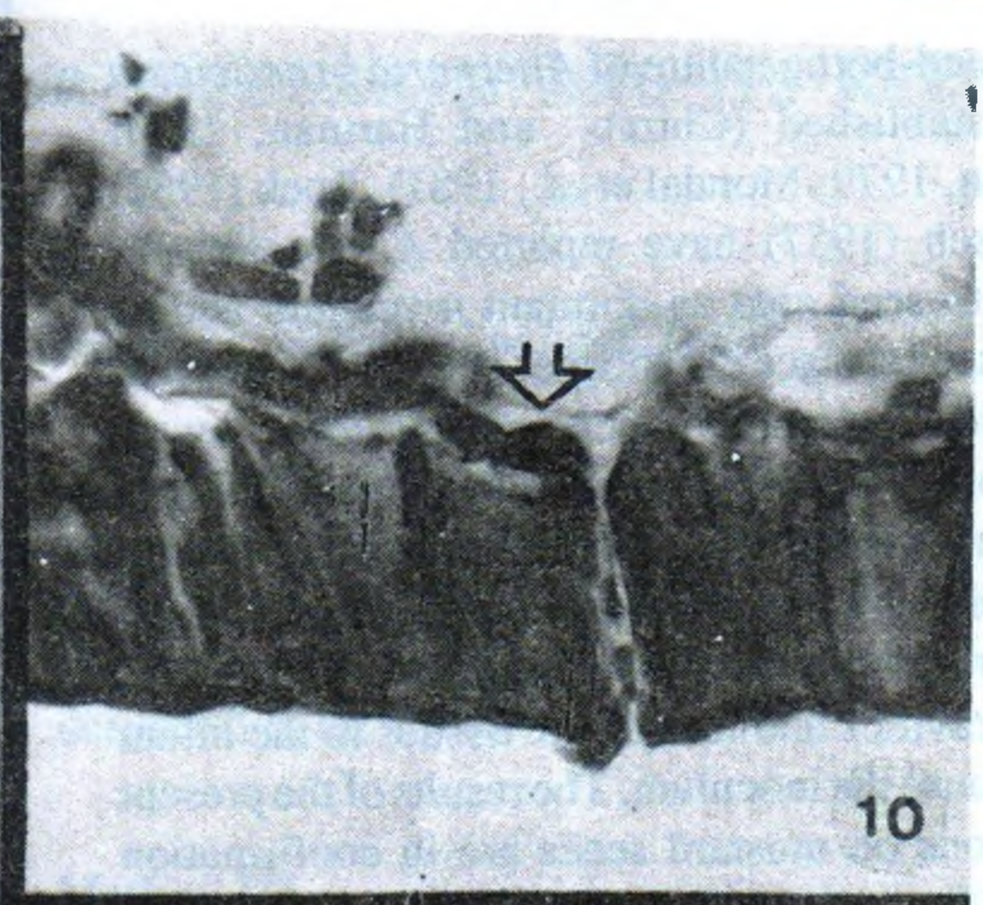
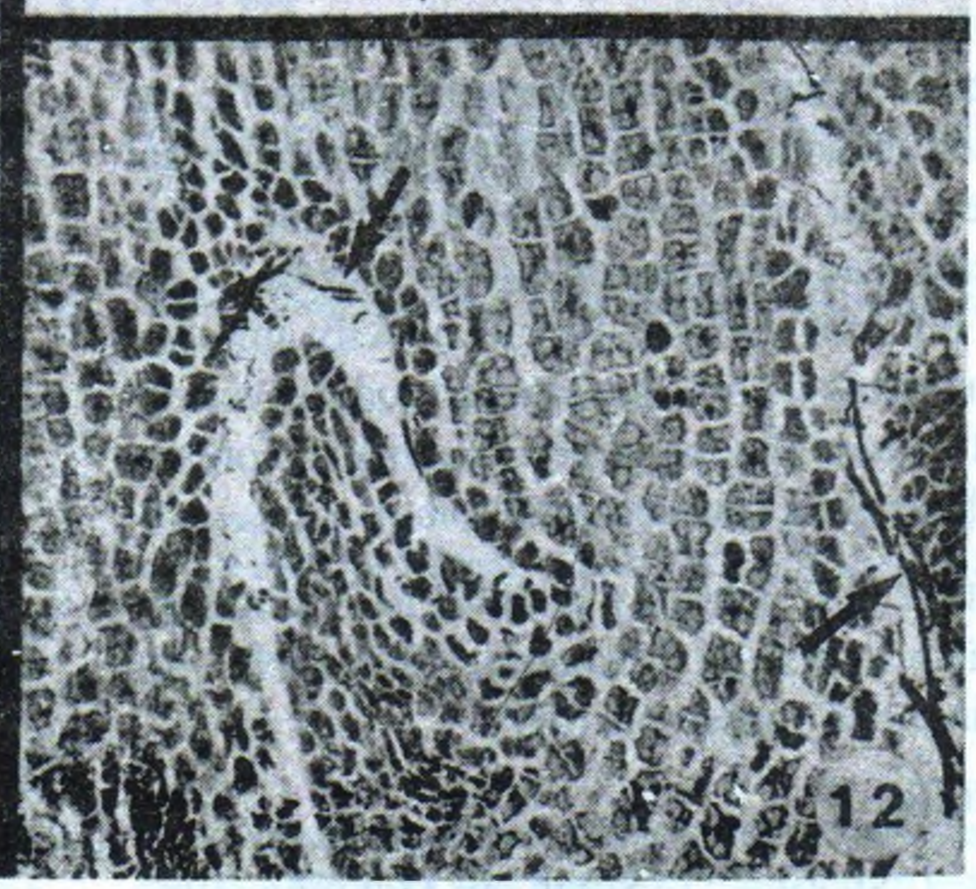


Figs. 1-8 Location of *Alternaria brassicicola* in mustard seeds.
1. Seed categories of infected sample : Bold - symptomless (upper), bold-discoloured (middle) and shrivelled-discoloured (lower) (X 12.5). 2. Conidia of *Alternaria brassicicola* (X 250). 3-6. Cleared wholemount preparations of epidermis of seed coat (Figs. 3,4), endosperm (Fig. 5) and cotyledon (Fig. 6) respectively showing inter-and intracellular mycelium(→) (X 125, X 125, X 125 and X 250) respectively. 7-8 Part of cross sections of bold-symptomless seed showing normal seed coat, endosperm and embryo in Fig 7 and of bold-heavily discoloured seed showing aggregation of mycelium (→) in seed coat epidermis and sub-epidermis. (X 125).

inter-as well as intracellular. In bold-discoloured seeds of sample nos. 2573, 2810 and 2929, 4,3 and 6 seeds in palisade layer; 3,2 and 4 seeds in endosperm and 2, 1 and 4 seeds in cotyledons respectively showed the







Figs. 9-12. Location of *Alternaria brassicicola* in shrivelled-discoloured seeds.
9. Part of longitudinal section of seed through hilum region showing course and aggregation of mycelium. X 125.
10. Part of seed coat showing mycelium (→) in palisade layer. Note weakened thickenings of its radial walls. X 500. 11. Part of embryo showing mycelium in spaces. X 250. 12. Part of seed through radicle showing intercellular mycelium (→). Also note the depletion of cell contents. X 500.

mycelium out of the 10 seeds examined. Seed coat epidermis in all the cases showed infection. In the bold mildly discoloured seeds the mycelium was confined to the seed coat only, but it colonised seed coat, endosperm and embryo in bold-heavily discoloured seeds. In the latter, cells of epidermis and subepidermis were distorted and mycelium penetrated the palisade layer (Figs. 7 and 8).

A heavy aggregation of mycelium was observed in seed epidermis of all the shrivelled-discoloured seeds examined. The epidermis showed prominent folds and its cells and those of subepidermis were distorted. The palisade cells revealed reduced thickenings of radial walls and penetration of mycelium at places (Figs. 9 and 10) into the endosperm. Out of the 10 seeds examined in each sample, 5,6 and 9 seeds in palisade layer; 5, 4 and 7 seeds in endosperm and 3,2 and 5 seeds in embryo showed mycelium in sample nos. 2573, 2810 and 2929 respectively. A heavy aggregation of mycelium was observed at the hilar region (Fig. 9), in the spaces between aleurone layer and cotyledons and in between the cotyledons (Fig. 11). The embryo in heavily infected seeds was reduced in size, occupying only a part of the space in seed and heavily colonised by the mycelium and the cells showed depletion of contents and necrosis (Fig. 12).

DISCUSSION

The seed-borne nature of *Alternaria brassicicola* is well established (Chirco and Harman, 1979; Richardson, 1979; Mondal *et al.*, 1981). Boek (1952) and Domsch (1957) have reported *A. brassicicola* restricted to seed coat as dormant mycelium and as spores on seed surface in cabbage. They concluded that this infection was unable to penetrate the deeper layers of seed coat apparently because of the thick-walled palisade layer forming a mechanical barrier whereas Knox-Davies (1979) reported that internal infection of the testa in cabbage seeds occurs largely at the hilum, while the cavities and fold of the tissues in the hilum area also house the inoculum. The results of the present investigations on mustard seeds are in confirmation with these findings. The pathogen was extraembryal and observed as spore contamination on seed surface as well as mycelium around the hilum region in epidermal cells of seed coat in bold-symptomless and bold-weakly discoloured seeds. In some seeds it spread to the other parts on seed surface. But in heavily infected seeds which included bold-discoloured and shrivelled-discoloured seeds, the pathogen heavily colonised inner parts becoming intraembryal.

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