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COLONISATION AND PHYTOPATHOLOGICAL EFFECTS OF *ALTERNARIA ALTERNATA* IN SEEDS OF FENUGREEK (*TRIGONELLA FONEUM-GRACEUM L.*)

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Due to the infection of *Alternaria alternata* 0.5 - 24.5% fenugreek seeds were brown discoloured. The incidence of fungus varied from 2 to 99% in seed lots. Both symptomless and symptomatic seeds carry infection of *A. alternata*. Infection was mostly localized in seed coat and occassionaly in endosperm and embryo. Meycelium was inter as well as intracellular. Heavy infection lead to partial or complete disintegration of mucilagenous endosperm. The fungus was found to be pathogenic causing blight symptoms in seedlings plants raised from artificially inoculated seeds.

Keywords : *Alternaria alternata* : Fenugreek seeds : Phytopathology.

Introduction

Fenugreek (*Trigonella foneum - graceum L.*) is an important spice, vegetable and fodder crop of Rajasthan. Seeds of fenugreek harbour predominatly *Cercospora traversiana*[1], *Alternaria alternata, Aspergillus* sp., *Fusarium* spp. *Rhizopus arrhizus* and *Rhizoctonia solani*[2,3]. *A. alternata* causing root of fenugreek has been first reported by Dwivedi *et al*[4]. Histopathology of *A. alternata* infected fenugreek seeds and host parasite relationship have not been carried out earlier hence the present study was undertaken.

Materials and Methods

One hundred sixty five seed samples collected from 18 districts of Rajasthan were subjeced to dry seed inspection and incubation methods[5]. For histopathology, seeds categorised as asymptomatic, weakly and heavily infected, of two samples carrying 97.5 and 99% infection of *A. alternata* were selected and studied by component plating, cleared wholemount preparation (50 seeds/ category) and micrtome sectioning (10 seeds/ category)[6].

Phytopathological effects of the fungus was studied using artificial seed inoculation method in petriplate and growing on test in pots[7]. The pathogen was isolated from naturally infected seeds and cultured in petriplates containing Potato Dextrose Agar (PDA). Healthy seeds pretreated with 0.5% aqueous solution of sodium hypochlorite were smothered on 15 days old sporulating cultures of the pathogen and plated on sterilized petriplate containing moisten blotters and also sown in pots containing sterilized soil. For control chlorine (0.5%) pretreated seeds were used. Data on percentage seed germination, symptoms and total loss were recorded.

Results and Discussion

Out of 165 samples, 84 samples carried 0.5 to 24.5% brown discolouration on seed coat (Fig. 1), which on incubation yielded *A. alternata*. Incubation on moistened blotters revealed 2 to 99% incidence of the pathogen. Chlorine pretreatment slightly knocked down the growth of pathogen (1.5-88%). The incidence of seed infection was found to be wide spread in Rajasthan with relatively higher incidence in samples from Jaipur and Nagaur.

Component plating of asymptomatic seeds revealed maximum of 30% infection of *A. alternata* on seed coat whereas symptomatic seeds showed 74%, 82% and 6 to 8% infection of seed coat, endosperm and embryo respectively of weakly infected seeds. In heavily infected seeds infection was 100%

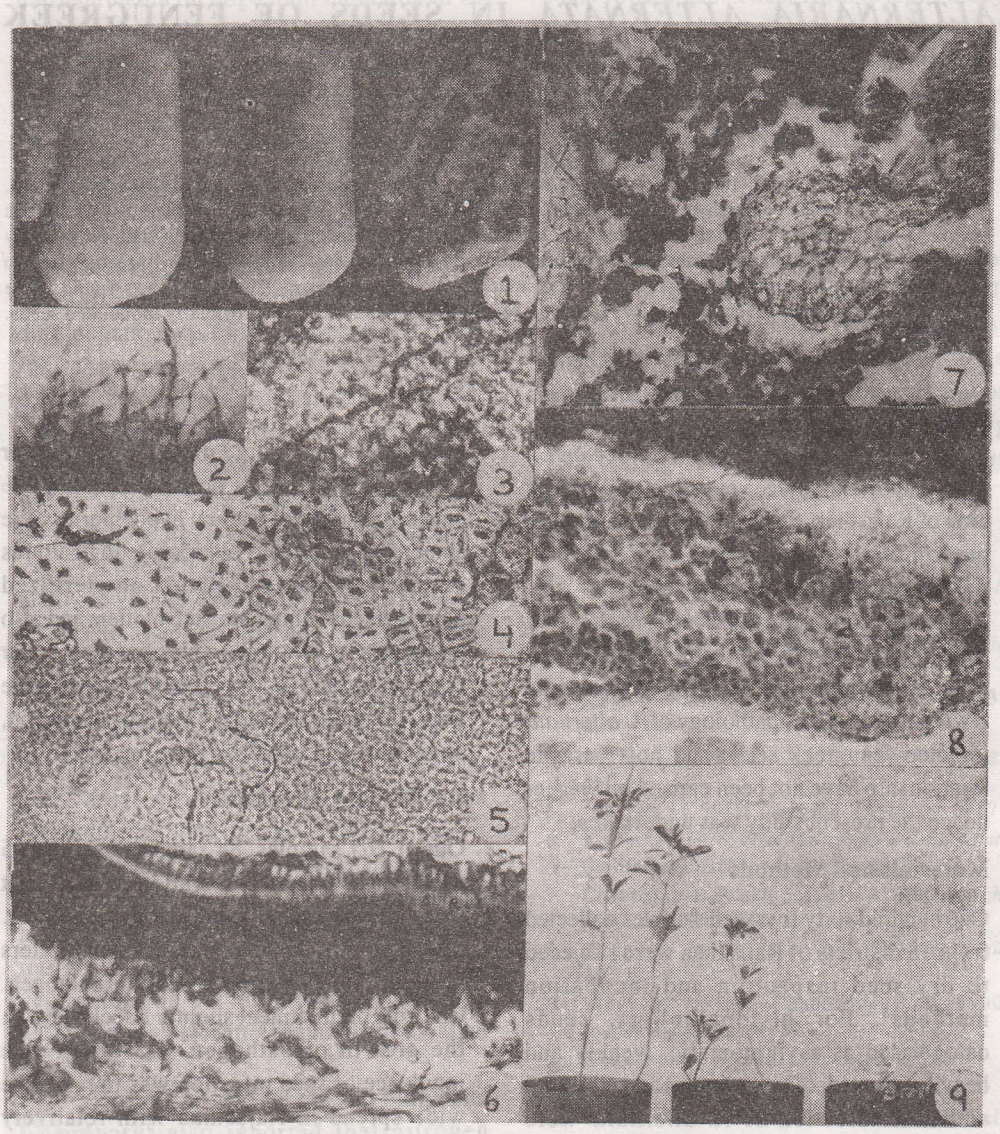

Fig. 1-9 Histopathology and phytopathological effects of *Alternaria alternata* in fenugreek. Fig. 1 - Fenugreek seed asymptomatic (left) weakly and heavily infected (right). (x-12.5); Fig. 2 - Incubated seed coat showing long conidial chains of *A. alternata*. (x 17.5); Fig. 3-5 - Cleared seed coat, endosperm and embryo respectively, showing branched, septate mycelium (x 125); Figs. 6-8 - Microtome sections (T.S.) of infected seeds; Fig. 6 - Heavily infected seed coat with mycelium in palisade, hourglass and parenchymatous cells (x 125); Fig. 7 - Mycelial infection and necrosis seen in hilar tracheids, stellate parenchyma (x 125); Fig. 8 - Cotyledon region of the embryo of heavily infected seed (x 125); Fig. 9 - Normal (control) and infected plants (right), note stunted growth, complete blighting and premature death of seedlings raised from artificially inoculated seeds.

in seed coat (Fig. 2), 60, 66% in endosperm and 34, 38% in embryo. Cleared wholemount preparations confirmed the results showing dark brown, septate, branched mycelium in different seed components (Figs. 3 to 5).

Anatomically fenugreek seed consist of seed coat, endosperm and embryo, which is composed of 2 large cotyledons, plumule and hypocotyl - radicle axis. The seed coat shows characteristic leguminous features viz. palisade cells, hourglass cells followed by 4-5 layers of parenchymatous cells. Endosperm comprises of aleurone layer and 7 to 8 layers of mucilagenous cells.

Seed coat of 3 (out of 10) asymptomatic seeds revealed mycelial infection, whereas 4 to 6 weakly infected seeds showed inter and intracellular mycelium in all the tissues of seed coat (Fig. 6). Dense mycelial mats and necrosis of tissues were seen in hilar region, stellate parenchyma and cells surrounding the tracheidal bar (Fig. 7). Endosperm and embryo were not infected. All seeds with heavy infection revealed dense growth of pathogen in seed coat with withered palisade cells and deformed hourglass cells. Necrosis and conidiogensis were also seen in tissues near hilar region. Endosperm of 2 to 5 seeds and embryo of 2 to 3 seeds showed dark brown as well as immature hyaline mycelium. The mucilagenous endosperm showed various degree of disintegration. Hyphae were also seen in a few outer layers of cotyledons (Fig.8) and the cells of radicle axis showed necrosis. The presnet study reveals that *A. alternaria* is internally seed-borne and intraembryonal pathogen. Presence of abundant mycelium, necrosis near hilar region, tracheidal bar and stellate parenchyma show that the fungus gains entry through hilar trachied. The presence of withered and loose palisade cells also indicate direct pentration through seed coat. Similar observations have been reported by Ilyas *et al*[8]., where they reported entry of *Cercospora kikuchi* through seed coat and hilar tracheids in seeds of soybean.

In artificial seed inoculation experiment, seed germination was 100% in control and 71 and 66% in petriplate and pot experiment, respectively. Small brown necrotic spots on basal leaves and stem appeared on 5th day in petriplates while on 22nd day after sowing in pots. The spots later coalesce to form large necrotic spots. Blight symptoms were recorded on 100% seedlings died before flowering, while in control seedling/plants flowering and pod formation occurred (Fig. 9). Incubation and cleared preparations of inoculated seeds and infected plants revealed presence of mycelium of *A. alternata* and it was isolated from all symptomatic parts of infected plants.

Though *A. alternata* considered to be a weak parasite, the results reveal that it is capable of causing typical blight symptom, premature death of seedlings and yield loss, behaving as an aggressive pathogen. Similar symptoms caused by the pathogen by artificial seed inoculation method have been reported by Singh and Chohan[9] in fenugreek and by Sharma[10] in *Eruca* and taramira seeds.

References

1. Richardson MJ 1990, *An Annotated List of Seed-Borne Diseases* IVth edition, proc. Int. Seed Test, Ass., Seed Test Ass., Wageningen, Netherlands
2. Hashmi M H 1988, *Pakistan* J. Bot. **20** 233
3. Sinha D C and Prasad R K 1989, Indian Phytopath. **42** 177
4. Dwivedi D K, Shukhla D N and Bhargava S N 1982, *Curr. Sci.* **51** 243
5. Anonymous 1976, *Seed Sci. & Technol.* **4** 48
6. Singh D, Mathur S B and Neergaard P 1977, *Seed Sci. & Technol.* **8** 85
7. Anonymous 1985, *Seed Sci. & Technol.* **13** 299
8. Ilyas M B, Dhingra O D, Ellis M A and Sinclair J 1975, *Plant Disease Reporter* **59** 17
9. Singh I and Chohan J S 1973, *Indian Phytopath.* **26** 749
10. Sharma N 1992, Ph. D. Thesis, University of Rajasthan, Jaipur, India.