

SUMMARY

- (1) A review of literature related to this investigation has been presented which deals mainly with serological relatedness among fungal species, cross reactive antigens and detection of pathogen in host tissues.
- (2) Materials used in this work and the methods followed for the experimentations have been discussed in details.
- (3) Meteorological data of 5 years 1996-2000 were collected which included maximum and minimum temperature, relative humidity (morning & evening) and monthly average rainfall from two different areas (Plains and Hills).
- (4) Disease incidence was found to be maximum from July to October in the Darjeeling hills where the average rainfall ranged between 449.022cms - 319.88cms, relative humidity 97% - 85%, maximum temp. ranging bet. 25°C - 22°C and the minimum temp. ranging bet 16°C - 12°C. Formation of heavy mist and fog also added to the severity of the blight.
- (5) Comparatively, the disease incidence was maximum from December to February in the Terai region. The average rainfall during this period was recorded between 15cms - 9 cms, RH being 95% - 92%, maximum temperature 25°C - 22°C & minimum temperature 12°C to 8°C, with the additional occurrence of mist and fog.
- (6) Pathogenicity of *E. vexans* was tested on 31 tea varieties (11 Darjeeling, 11 Tocklai and 9 UPASI) were tested. AV2, TV18 and UP 8 were found to be most susceptible.
- (7) Polyspecific antisera were raised against antigen preparations from blister infected tea leaves of Hansqua Tea Estate (plains) and Castleton Tea Estate (Darjeeling hills).
- (8) Polyclonal antibody was raised against *E. vexans* basidiospores, collected from blister infected tea leaves of Castleton Tea Estate.
- (9) In agar gel double diffusion tests anti-*E. vexans* antiserum was cross reacted with leaf antigens of all 31 tea varieties. Strong precipitin reaction occurred in homologous reactions and in reactions involving susceptible varieties while, no precipitation occurred with resistant varieties, non hosts or non pathogens.
- (10) All the antisera raised, were purified by ammonium sulphate precipitation and DEAE cellulose chromatography. IgG, obtained in each case was used for ELISA tests.
- (11) Optimization of antisera were determined by DAC-ELISA. An antiserum dilution of (1:250) and an enzyme (alkaline phosphatase) dilution of 1:10,000 were optimum for polyspecific as well as polyclonal antisera.

(12) Cross reactive antigens of all the 31 tea varieties (healthy) were tested with anti-*E. vexans* antiserum and found out that AV2, TV18, TV27, UP8 & BS/7A/76 showed very high absorbance. Reciprocal cross reactions with antisera raised against TV18 and CP1 and non pathogen *Fusarium graminearum* showed high absorbance values in susceptible reactions and low in resistant reactions as well as reactions with non host and non pathogen.

(13) *E. vexans* was detected in artificially inoculated leaves of 14 tea varieties on basis of significantly higher absorbance values in ELISA of inoculated extracts as compared to healthy extracts using anti-*E. vexans* antiserum. Similarly natural infection could also be detected by ELISA.

(14) *E. vexans* infection was detected as early as 48h. after inoculation in susceptible varieties like AV2 & TV18 by DAC-ELISA. Highest absorbance values were noted 12 days after inoculation.

(15) Callus was induced from stem segments of TV18 and from this callus loosened cells were obtained. Immunofluorescence studies of anti-*E. vexans* antiserum revealed bright fluorescence after treatment with FITC.

(16) Cross sections of healthy tea leaves (TV18 and AV2) treated with anti-*E. vexans* antiserum and then with FITC, developed a bright fluorescence throughout, which showed high CRA with *E. vexans*.

(17) Cross-sections of infected tea leaves treated with homologous anti-*E. vexans* antiserum showed bright fluorescence in palisade and spongy parenchymatic tissues (area of infection). Basidiospores of *E. vexans* treated with homologous anti-*E. vexans* antiserum and FITC showed bright fluorescence.

(18) *E. vexans* in healthy artificially inoculated tea leaves as well as naturally infected tea leaves were also observed by Dot blot immunoassay and western blotting technique.

(19) Field management of blister blight after spray of systemic fungicide (Hexaconazole) and foliar biocides, was evaluated by percentage disease incidence.

20. Polyclonal antibody raised against basidiospores of *E. vexans* was used for DAC-ELISA formats. It was found that the absorbance (A 405) values always reduced in treated leaf tissues than untreated ones.