Part X : Analysis of antigenic pattern after chemical induction of resistance in tea to *B. carbonum*

It is evident from the results of immunodiffusion and immunoelectrophoretic tests (vide part VIII) as well as indirect enzyme linked immunosorbent assay (vide Part IX) that a serological relationship exist between susceptible tea varieties and isolates of *B. carbonum*. It is also evident from the results (vide Part VI) that among the various chemicals tested nickel chloride and nickel nitrate reduced disease symptoms markedly in susceptible tea variety (TV-18). Consequent changes in the levels of antimicrobial compounds were also detected. Hence it was decided to study changes in the antigenic pattern in treated plants. To determine this, susceptible tea plants (TV-18) were treated separately with nickel chloride (10^{-5}M) or nickel nitrate (10^{-5} M) as described in materials and methods. Control (healthy) plants were sprayed with distilled water. Antigens were extracted from untreated and treated young tea leaves three days after third spray with above chemicals. Antigens of untreated and treated with nickel chloride and nickel nitrate leaves of susceptible tea variety (TV-18) and *B. carbonum* were cross reacted with antisera of *B. carbonum* (BC1A) and tea leaves (T18A). Results are presented in Table 42. In agar gel double diffusion test in this case also common precipitin bands were detected when antiserum of TV-18 was cross reacted with antigens

of B. carbonum. (Plate-XII ; fig.1). Reciprocal cross reaction with the antiserum of B. carbonum and antigens of TV-18 gave common precipitin band. An interesting observation was made when antiserum of TV-18 was cross reacted with the antigen preparations of healthy and treated leaves of the same variety. Strong precipitin bands were evident in the homologous reaction between antigens and antisera of TV-18. But antigen prepared from nickel chloride treated leaves developed very faint diffused band in this reaction and nickel nitrate treated leaves exhibited weak precipitin bands (Plate XII, fig.2). Reciprocal cross reactions between antisera of B. carbonum and antigens of nickel chloride treated leaves of TV-18 failed to develop any precipitin bands.

To confirm this, immunoelectrophoretic tests was performed. Results are given in Table-43. B. carbonum showed precipitin arcs in homologous reactions. In the cross reaction between antigens of untreated leaves of susceptible variety (TV-18) with antiserum of B. carbonum 2 precipitin arcs developed But when antiserum of B. carbonum was cross reacted with the antigens of nickel chloride treated leaves of TV-18 only one precipitin arc was evident (Plate XII , figs. 3 & 4). It can be explained that untreated healthy leaves of TV-18 was antigenically close to B. carbonum sharing 2 of the 5 antigenic constituents of the latter, while nickel chloride treated leaves of TV-18 shared only 1 ofthe 5 antigenic constituents of

Table 42 : Antigenic relationship between isolate of *B. carbonum* (BC1) and susceptible tea variety (TV-18) before and after treatment with chemicals

Antigens of host and parasite	Antisera of TV - 18 (T18A)
B. carbonum	+
Susceptible variety (TV-18)	
Untreated	+
Treated with nickel nitrate[a]	±
Treated with nickel chloride[a]	±

Antigens of host and parasite	Antisera of *B. carbonum* (BC1A)
B. carbonum	+
Susceptible variety (TV-18)	
Untreated	+
Treated with nickel nitrate[a]	±
Treated with nickel chloride[a]	-

precipitin band present (+), precipitin band absent (-).
weak precipitin band (±).

[a] plant treated with 10^{-5}M nickel nitrate or nickel chloride.

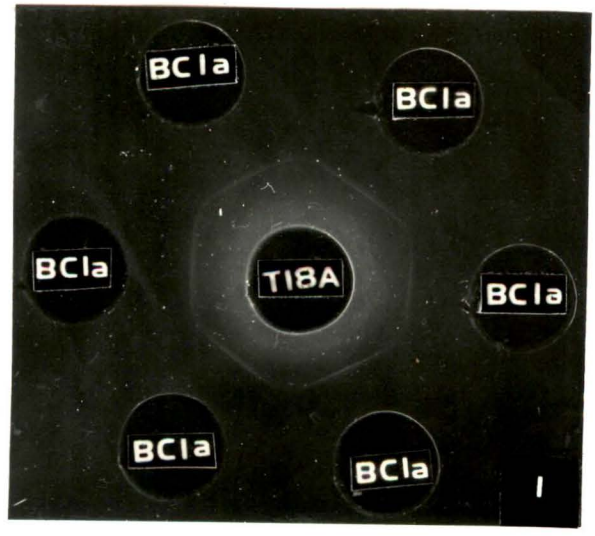
Table 43 : Antigenic comparison of B. carbonum (BC1) and susceptible tea variety (TV-18) before and after treatment with nickel chloride

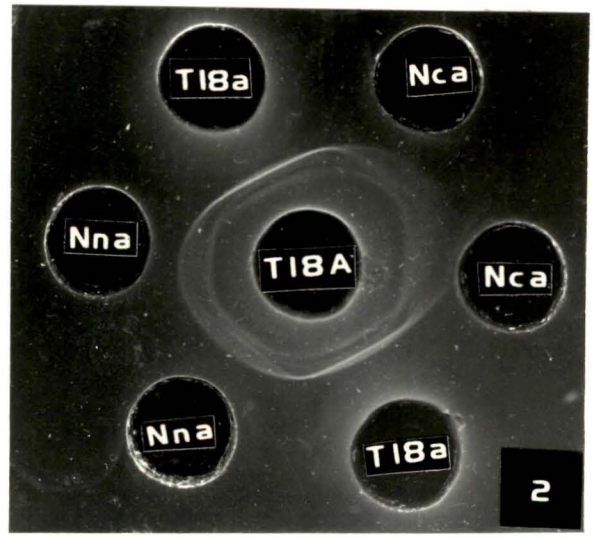
Antigens of host and parasite	Antiserum of B. carbonum (BC1A)					
	Precipitin lines					
	1	2	3	4	5	6
B. carbonum (BC1a)	+	+	+	+	+	-
Susceptible variety (TV - 18)						
Untreated	-	+	-	-	+	-
Treated[a]	-	-	-	-	+	-
Antigens of host and parasite	**Antiserum of TV-18 (T18A)**					
	Precipitin lines					
	1	2	3	4	5	6
Susceptible variety (TV - 18)						
Untreated	+	+	+	+	+	+
Treated [a]	+	-	-	+	+	+
B. carbonum	-	+	-	-	+	-

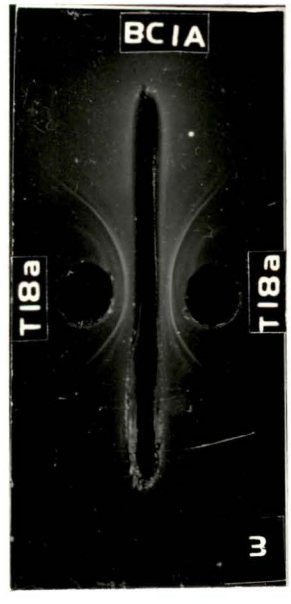
a Plants treated with 10^{-5} M Nickel chloride

Precipitin line present (+) ; precipitin line absent (-)

PLATE - XII (figs. 1 & 2)-Agar gel double diffusion(figs. 3 & 4)-Immunoelectrophoretic tests using antigens and antisera of host and parasite. fig. 1-Central well contained antiserum of TV-18 (T18A) and surrounding wells contained antigens of B. carbonum isolates BC1 (BC1a) fig. 2 - Central well contained antis erum of TV-18 (T18A) and surrounding wells contained antigens of untreated leaves of TV-18 (T18a), nickel nitrate treated leaves of TV-18 (Nna) and nickel chloride treated leaves of TV-18 (Nca). fig. 3- Central rectangular trough contained antiserum of B. carbonum (BC1A) and the wells contained antigens of TV-18 (T18a) Fig. 4-central rectangular trough contained antiserum of B. carbonum(BC1A) and the wells contained antigens of nickel chloride treated leaves of TV-18 (Nca).


BC1a
BC1a
BC1a
T18A
BC1a
BC1a
BC1a
1


T18a
Nca
Nna
T18A
Nca
Nna
T18a
2


BC1A
T18a
T18a
3

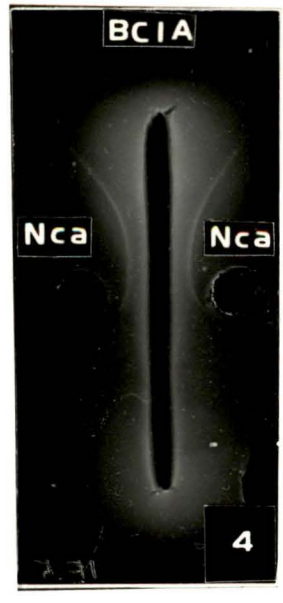

BC1A
Nca
Nca
4

PLATE -XII

B. carbonum. Similarly antigen of untreated and nickel chloride treated tea leaves (TV-18) and B. carbonum (BC1) were cross reacted with antiserum of TV-18. In the homologous reactions TV-18 exhibited 6 precipitin arcs. Nickel chloride treated leaves of TV-18 shared only 4 of the 6 antigenic constituents of untreated leaf.

Part XI : Studies on the tissue and cellular location of major cross reactive antigens shared by Camellia sinensis and Bipolaris carbonum

Fluorescent antibody labelling with fluorescein isothiocyanate (FITC) is known to be one of the powerful techniques to determine the cell or tissue location of major cross reactive antigen shared by plant host and parasite. In the present study, immunodiffusion and immunoelectrophoretic tests as well as indirect enzyme linked immunosorbent assay clearly indicated the presence of major cross reactive antigenic substance (CRA) common to C. sinensis and B. carbonum. It was decided to determine the tissue and cellolar location of CRA in tea leaves.

Antibodies indirectly labelled with fluorescein isothiocyanate (FITC) were used to determine the location of CRA in sections of tea leaves (TV-18) and fungal cells (B.carbonum).