EXPERIMENTAL

In the present study experiments were conducted to determine the biochemical responses of tea plants to various stresses i.e. abiotic, biotic and anthropogenic. In abiotic stress plants were subjected to both elevated temperatures and drought separately. In case of elevated temperatures whole plant could withstand temperature of only up to 50^0C in control condition after which the leaves become scorched and dried. This was more apparent in Darjeeling Tocklai varieties whereas the UPASI varieties did not exhibit such scorching.

The response of different tea varieties to water stress also varied. Water stress was applied up to 12 days at an interval of 4 days to six selected varieties. It was observed that severe wilting symptom were exhibited by TV and Darjeeling varieties whereas the UP varieties were physically more drought resistant with wilting symptom not as pronounced as in the other two cases (Plates IV and V). The percentage of soil moisture, determined after every period of drought, revealed that after 12 days of drought, the moisture content had decreasd from approx.98% in control to 38%.Morphological aand physiological changes noted in the Tocklai, UPASI and Darjeeling varieties are depicted in Table 1.

Table 1: Physiological and morphological changes in tea plants subjected to drought

Tea varieties	Days of water stress			
	0	4	8	12
Tocklai	Leaves green turgid	Starting of some wilting symptoms	Excessive curling; stunted growth	Extreme wilting
UPASI	Leaves green, turgid	Healthy; no wilting	No wilting symptoms	Starting of some wilting symptoms
Darjeeling	Leaves green turgid	Starting of some wilting symptoms	Excessive curling and browning of leaves	Extreme wilting

Since spraying with insecticide and fungicide is commonly done in the tea gardens an insecticide (Monocil) and a fungicide (Hexaconazole) were taken as an anthropogenic stress inducers. Spray schedule as mentioned in the materials and methods was followed and it was observed that from soon after the 4th spray the leaves of the seedlings exhibited scorching like symptoms and turned black. Hence

Plate IV (figs. A-D) : Potted tea plants subjected to drought;A& C- Healthy; B&D –Treated

Plate V (figs. A-D) : Potted tea plants subjected to drought; A& B- Healthy; C&D – Treated

biochemical analyses were done only till the third spray. Such symptoms were more prominent in case of hexaconazole.

Blister blight infected samples were mainly collected from different tea gardens from Darjeeling hills as described previously. The diseases being prevalent in the hill areas, most of the samplings were done from such area. In Dooars blister blight was found to appear in one of the gardens from where the samplings were done. Symptoms were most common on the young leaves and in the early stages of infection pink translucent spot was observed on the ventral side of the leaves which gradually changes to white full grown blister and then in the last stages dried up and turned black (Plate VI). Since the pathogen is an obligate one and it cannot be grown in culture for artificial inoculation spores were used and inoculation was done as described. Visible symptom appeared after 19 days of inoculation and the nature of blister were different from that of natural infection (Plate VII). Such artificially inoculated leaves were taken for certain biochemical analysis in order to compare with the naturally occurring blister infection. In the following pages results of all experiments have been presented.

4.1. Phenylalanine ammonia lyase activity of tea leaves under different stresses

Activity of phenylalanine ammonia lyase was assayed in different tea varieties subjected to abiotic, anthropogenic and biotic stress as described in the materials and methods.

4.1.1. Abiotic stress

For abiotic stress, elevated temperature and drought were considered, and PAL activity was assayed in tea leaves subjected to these stresses. Results are presented below.

4.1.1.1. Elevated temperature

Elevated temperature treatment was applied both on detached leaves as well as on young intact seedlings. For detached leaves, seven UPASI varieties (UP-2, UP-3, UP-8, UP-9, UP-26, BSS-2, BSS-3), seven Tocklai varieties (T-17/1/54, TV-9, TV-18, TV-22, TV-23, TV-25, TV-26) and six Darjeeling varieties (BS/7A/76, T-78, B-157, T-135, AV-2, HV- 39) were selected. Leaves were subjected to elevated

Plate VI (figs. A & B) : Natural blister infection in tea leaves; A- Portion of shoot ; B- Detached leaves

Plate VII (figs. A-C): Tea plant (TV-26) artificially inoculated with spores of *Exobasidium vexans* showing symptoms 19 days after inoculation; A- whole plant;B&C-Blister infected leaf (enlarged views)

temperature up to 60^{0}C. Results revealed that in all Tocklai varieties there was an initial increase in PAL activity up to 45^{0}C after which activity declined. In all of the Darjeeling and most of the UPASI varieties activity declined from 40^{0}C onwards (Table2, Fig.1).

Table 2: Effect of elevated temperature on PAL activity in tea leaves

Varieties	PAL activity (µg cinnamic acid/ g tissue/ min) Temperatures (^{0}C)					
	Control	40	45	50	55	60
UP-2	432±9.88	368±9.16	305±8.09	282±9.20	275±7.08	255±11.1
UP-3	448± 6.16	440±7.18	410±1.14	383±11.9	340±6.99	270±8.22
UP-8	455± 9.98	434±8.67	325±8.26	295±8.10	270±6.67	242±9.39
UP-9	310± 5.10	325±7.88	392±11.0	290±20.9	266±5.39	255±9.46
UP-26	397±7.69	410±7.25	440±9.96	385±11.6	360±11.5	282±8.11
BSS-1	211±5.12	202±9.12	140±9.29	123±9.08	109±5.04	089±8.01
BSS-2	221±8.01	212±10.3	199±6.12	156±8.08	121±13.0	101±8.02
T-17/1/54	433±13.9	420±11.6	400±12.0	365±3.91	350±14.6	320±8.96
TV-9	320±8.09	376±6.18	432±2.22	425±6.16	385±7.15	225±7.15
TV-18	368±.7.29	372±8.89	447±4.14	432±8.67	425±7.16	385±9.15
TV-22	334±7.16	340±12.8	397±4.28	360±4.14	334±9.08	290±7.12
TV-23	282±5.79	292±5.62	385±8.98	355±6.09	352±26.1	165±9.55
TV-25	330±5.15	337±6.22	425±4.10	405±8.68	385±17.5	300±20.8
TV-26	265±7.59	367±8.73	405±6.44	400±8.29	355±13.7	300±11.5
BS/7A/76	225±09.9	190±8.91	170±16.4	147±6.76	117±6.87	89±23.08
T-78	230±2.19	185±9.49	141±10.0	126±6.29	116±9.09	101±6.13
B-157	195±8.12	175±8.19	170±8.42	135±8.19	108±7.58	076±9.95
T-135	143±9.01	139±8.12	136±8.08	116±8.05	108±9.10	097±7.03
HV-39	205±6.12	148±7.08	105±8.09	090±7.02	065±6.02	040±9.01
AV-2	196±8.16	136±11.2	129±6.19	110±7.02	089±11.9	062±10.0

Effect of elevated temperatures on PAL activity in tea leaves

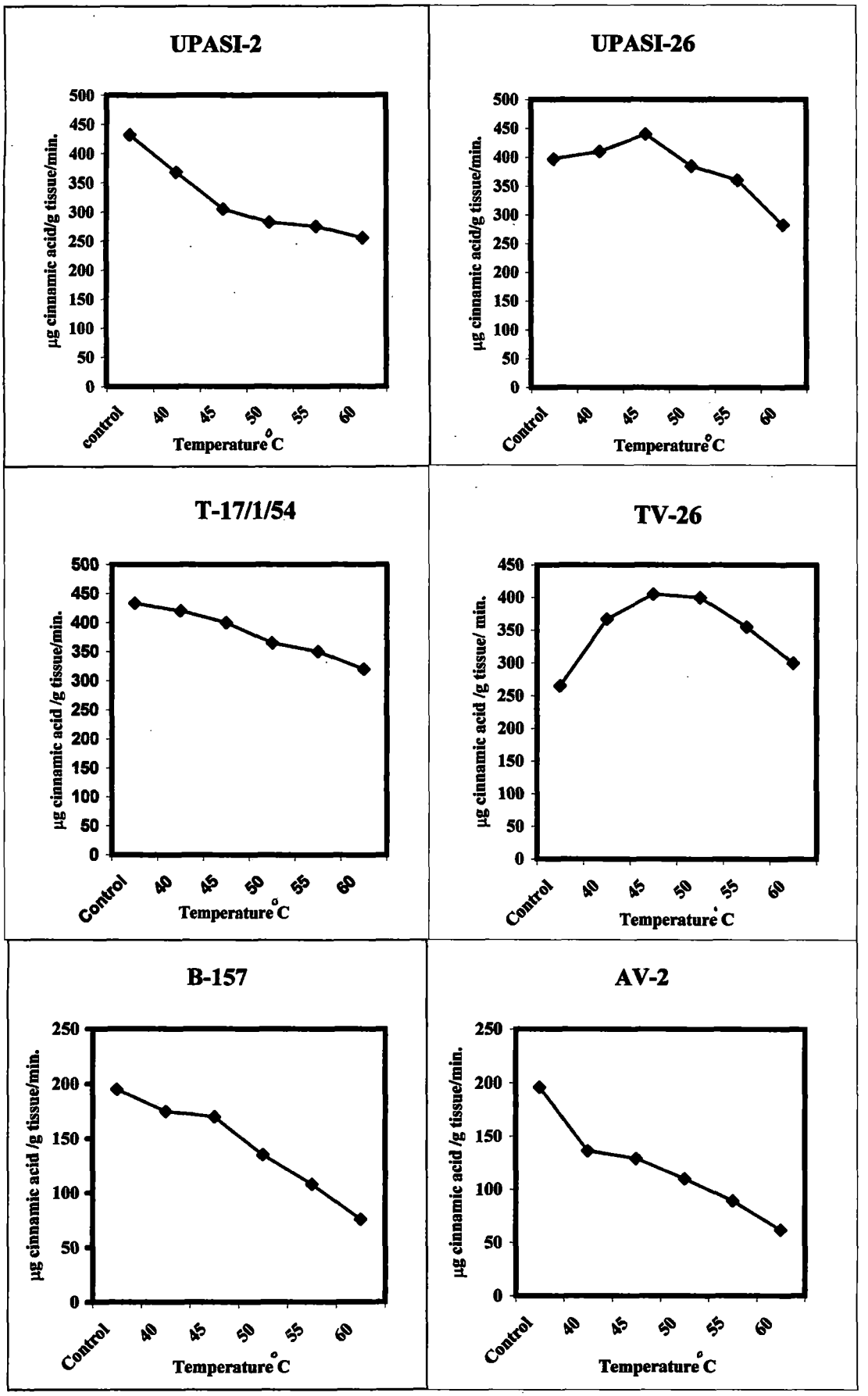


Fig.1

Table 2a: Analysis of variance of data presented in Table 2

Source	D.F.	S.S.	M.S.	F	C.D. (5%)
Treatment	5	160423.2	32084.64	20.2128	24.9963
Varieties	19	1391248.0	73223.58	46.1297	
Error	95	150797.5	1587.34		
Total	119	1702469.0			

Of the twenty varieties considered for detached leaf treatment, seedlings of ten varieties (UP-3, UP-9, BSS-2, T-17/1/54, TV-18, TV-22, TV-25, TV-26, AV-2, HV-39) were subjected to elevated temperature trteatment. In this case it was observed that temperature treatment of only up to 50^0C could be given since higher temperature led to scorching of the plants. Out of the ten varieties, seven varieties showed a decline in activity from 40^0C onwards. In TV-23, TV-25 and UP-9 there was an increase at 40^0C before activity declined (Table 3, Fig.2). In this case, Differences in PAL activity were significantly different at 45 and 50^0C, but not at 40^0C. Significant differences were also obtained between activities at 40^0C with those at 45 and 50^0C. In general, differences in activity of PAL were significantly different from control from 50^0C onwards.

Results of the effect of the elevated temperatures on PAL activity revealed that the optimum temperature in case of Tocklai varieties range from 40^0C - 45^0C where as in the Darjeeling varieties all higher temperatures resulted in a decline in activity.

4.1.1.2. Drought

In all of the six varieties subjected to water stress for 4, 8 and 12 days PAL activity registered an initial significant increase at 4 days after which activity declined steadily, to register a significantly lower value at 12 days drought (Table 4. Fig. 3).

Effect of elevated temperatures on PAL activity in tea seedlings

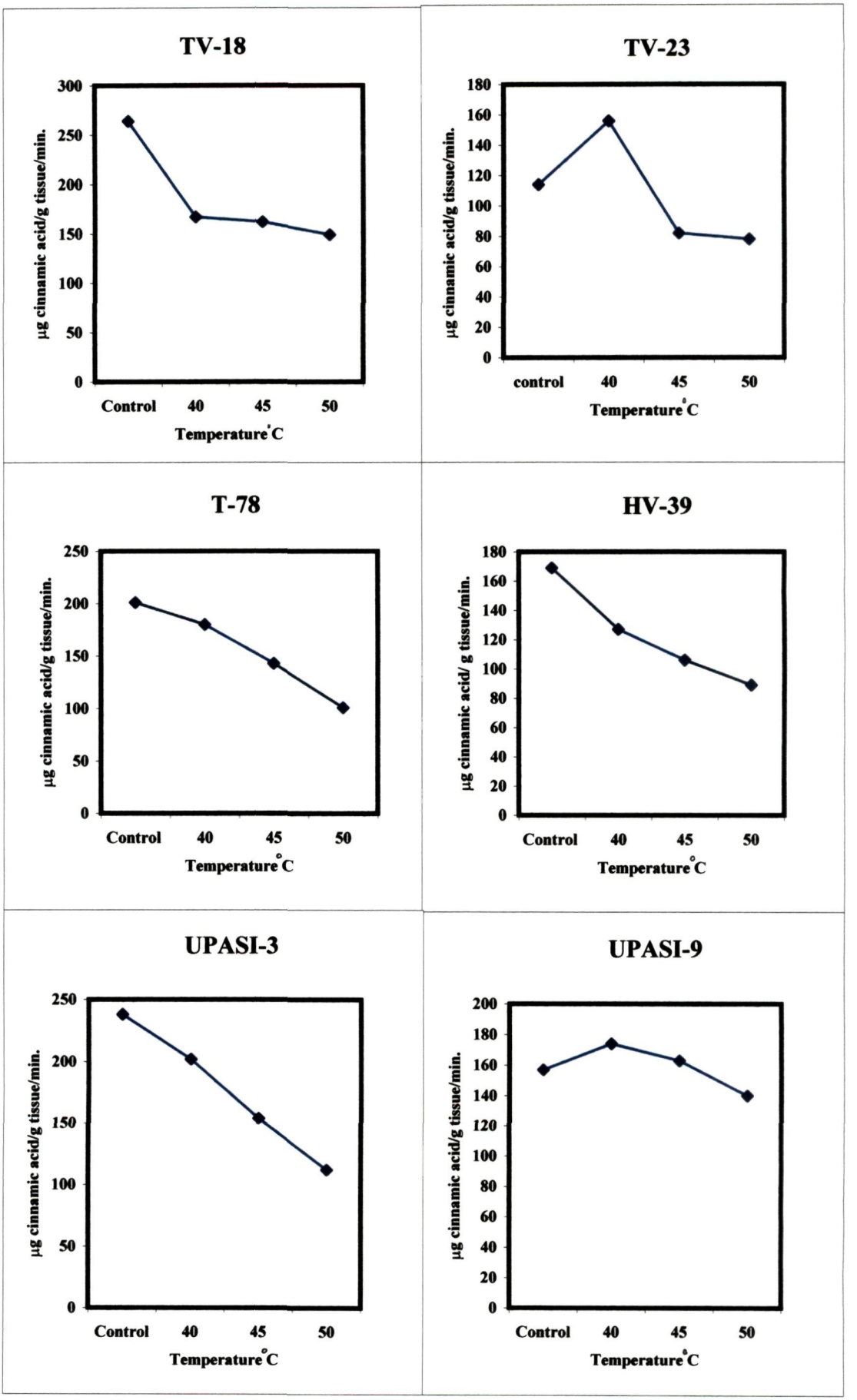


Fig.2

Changes in PAL activity of tea seedlings subjected to water stress

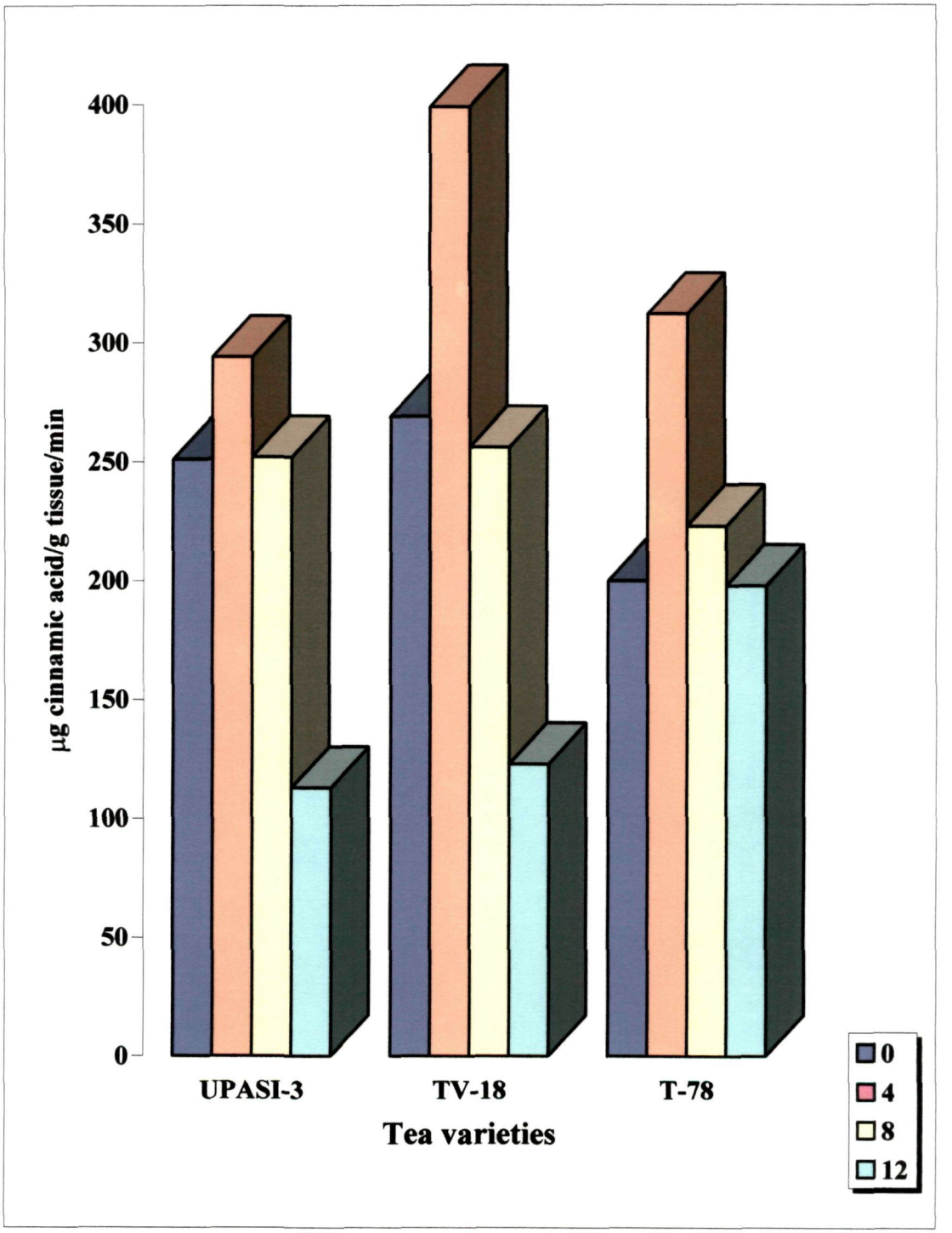


Fig.3

Table 3: Effect of elevated temperatures on PAL activity in young tea seedlings

Varieties	PAL activity (µg cinnamic acid /g tissue /min.) Temperature (^{0}C)			
	Control	40	45	50
T-17/1/54	218 ± 12.8	212±18.6	209±23.2	200±21.6
TV-18	264 ± 17.6	167±16.3	162±27.6	149±5.8
TV-23	114 ± 21.3	156±21.8	082±12.6	078±14.6
TV-25	121 ± 14.1	142±14.1	089±10.6	069±23.1
TV-26	262±14.8	216±14.6	192±12.3	141±9.08
T-78	201±19.8	180±18.9	143±21.4	101±8.12
HV-39	169±21.6	127±27.6	106±13.3	089±14.6
UPASI-3	238±9.06	202±16.2	154±16.2	112±12.1
UPASI-9	157±9.68	174±11.4	163±16.1	140±14.1
BSS-2	150±9.33	123±16.6	113±18.8	107±12.8

Table 3a: Analysis of variance of data presented in Table 3

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	3	29178.57	9726.19	18.4338	24.3767
Varieties	9	53851.88	5983.54	11.3405	
Error	27	14245.95	527.62		
Total	39	97276.40			

4.1.2. Anthropogenic stress

In the present study, young potted plants of two varieties TV-18 and TV-26, were selected for application of anthropogenic stress, i.e. spraying with a fungicide and insecticide.

4.1.2.1. Fungicide

Hexaconazole was sprayed at regular intervals as described in materials and methods. PAL activity was assayed after each spray and results are presented in Table 5. In both varieties PAL activity declined significantly after each spray in

relation to control. The difference of treatment with control was statistically significant from 2nd spray onwards .

Table 4: Effect of water stress on PAL activity on young tea seedlings

Tea variety	PAL activity (μg cinnamic acid /g tissue /min.) Days of water stress			
	Control	4	8	12
TV-18	269 ± 12.0	399 ± 11.6	256 ± 23.2	123 ± 9.08
TV-26	264 ± 22.9	356 ± 18.5	208 ± 13.4	117 ± 12.6
UPASI-3	251 ± 21.1	294 ± 23/2	252 ± 13.7	113 ± 29.6
UPASI-26	178 ± 13.7	280 ± 18.4	166 ± 14.5	120 ± 12.9
T-78	200 ± 18.8	312 ± 12.2	223 ± 11.6	198 ± 15.6
HV-39	186 ± 11.1	208 ± 10.1	254 ± 13.2	120 ± 18.5

Table 4a: Analysis of variance of data presented in Table 4.

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	3	93527.46	31175.8	19.7259	57.15174
Varieties	5	15810.38	3162.07	2.0007	
Error	15	23706.79	1580.45		
Total	23	133044.6			

Table 5. Effect of fungicide on PAL activity on young tea seedlings

Tea varieties	PAL activity (μg cinnamic acid /g tissue /min.) Control	1st spray	2nd spray	3rd spray
TV-18	271 ± 13.6	255 ± 11.2	196 ± 13.2	179 ± 13.5
TV-26	278 ± 14.2	210 ± 12.1	187 ± 11.8	162 ± 13.2

C.D.(5%) of treatment mean effects = 64.24348

4.1.2.2. Insecticide

PAL activity was observed to decline after spraying with monocil. After the third spray PAL activity was almost half of that of control (Table 6). The activities were significantly lower from 2nd spray onwards.

Table 6: Effect of insecticide on PAL activity on young tea plants

Tea varieties	PAL activity (μg cinnamic acid /g tissue /min.)			
	Control	1st spray	2nd spray	3rd spray
TV-18	271 ± 13.6	240 ± 14.3	129 ± 16.9	091 ± 9.08
TV-26	278 ± 14.2	251 ± 13.7	126 ± 18.1	120 ± 10.5

C.D.(5%) for treatment mean effects = 39.47001

Thus both fungicide and insecticide spray led to an overall decrease in PAL activity in both of the tested varieties, which was significant in case of 3rd and 4th spray (Fig. 4).

4.1.3. Biotic stress

Healthy and blister infected leaves from six tea gardens in the Darjeeling region and one from the Terai were collected and PAL activity assayed. In all cases, significant decrease in activity was obtained in leaves following infection (Table 7, Fig.4).

Table 7. Effect of blister blight infection on PAL activity of tea leaves

Tea Gardens	PAL activity (μg cinnamic acid /g tissue /min.)	
	Healthy leaves	Infected leaves
MarH TG	408 ± 21.6	276 ± 12.9
Dil TG	552 ± 19.7	294 ± 13.6
Sin TG	696 ± 20.2	264 ± 16.7
Cas TG	480 ± 17.5	180 ± 14.4
Mok TG	540 ± 16.6	302 ± 12.9
Mar TG	420 ± 12.2	238 ± 21.4
Has TG	448 ± 11.0	396 ± 21.6
Art.ino.	154 ± 9.4	068 ± 12.1

C.D.(5%) for treatment mean effects = 124.47820

Effect of anthropogenic and biotic stress on PAL activity in tea leaves

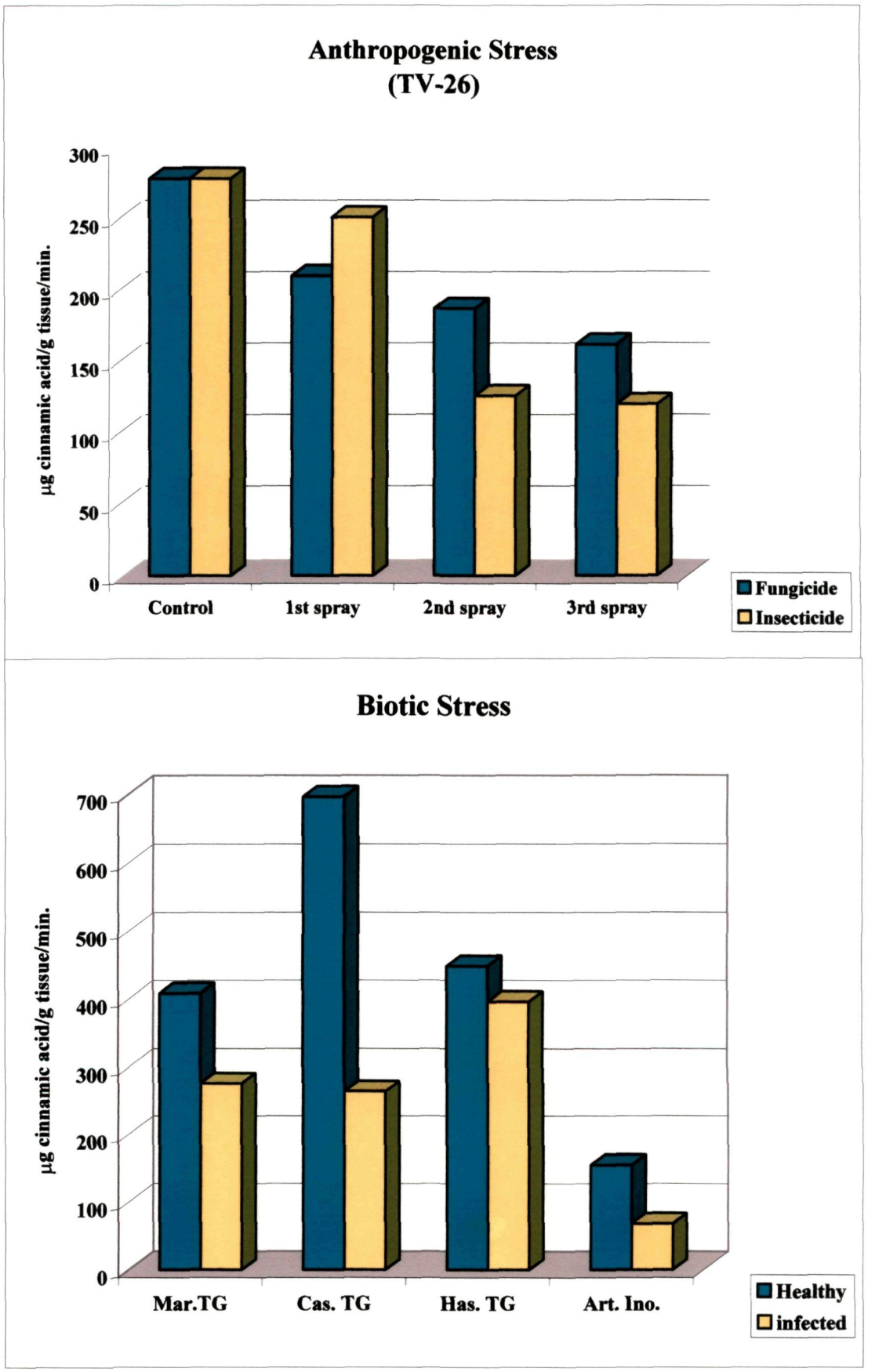


Fig.4

Leaves were also artificially inoculated with spores of E.*vexans* to determine PAL activity during the appearance of symptom. Artificial inoculation also led to a decrease in activity, which was highly significant in case of the first leaf, where the symptom of blister had appeared (Table 7). In the other leaves which had been inoculated (2nd, 3rd and 4th) but where no symptom were developed, activity was either not lesser than the healthy ones (2nd leaf) or only slightly lesser (3rd and 4th).

Results of all experiments relating to PAL activity has shown that in general activity declined following stresses. But in certain cases (elevated temperature up to 40^0C or 4th day water stress) plants initially responded by an increase in activity before declining. Constitutive PAL activity among the varieties also differed. Age of the plant seemed to affect PAL activity markedly as the leaves from the older plants showed higher activity (more than 400 µg cinnamic acid) whereas the seedlings showed the values even as low as 100µg cinnamic acid. Thus PAL activity in the plants was constitutively regulated by the varieties, age of the plants, types of stress etc.

4.2. Polyphenol oxidase activity of tea leaves under stress

PPO is an important enzyme in the phenol metabolism of tea plants and plays a major role in tea fermentation. Hence the effect of various stresses on activity of PPO was determined and the results have been presented below:

4.2.1. Abiotic stress

4.2.1.1. Elevated temperature

PPO was extracted and assayed in different tea varieties as described in materials and methods. In the Tocklai and UPASI varieties subjected to elevated temperature up to 60^0C,in the detached leaves, optimum temperature range from 45^0C-50^0C whereas in the Darjeeling varieties the optimum was 40^0C(Table8, Fig.5). The differences in activity at elevated temperatures were significantly different from that of control, even at 40^0C. At 60^0C, in all varieties, the activity was very low. In case of intact plants eight of the ten varieties showed an optimum temperature at 40^0C whereas in two varieties (HV-39, UP-3) the optimum was at 45^0C (Table 9, Fig. 6).

Effect of elevated temperatures on PPO activity in tea leaves

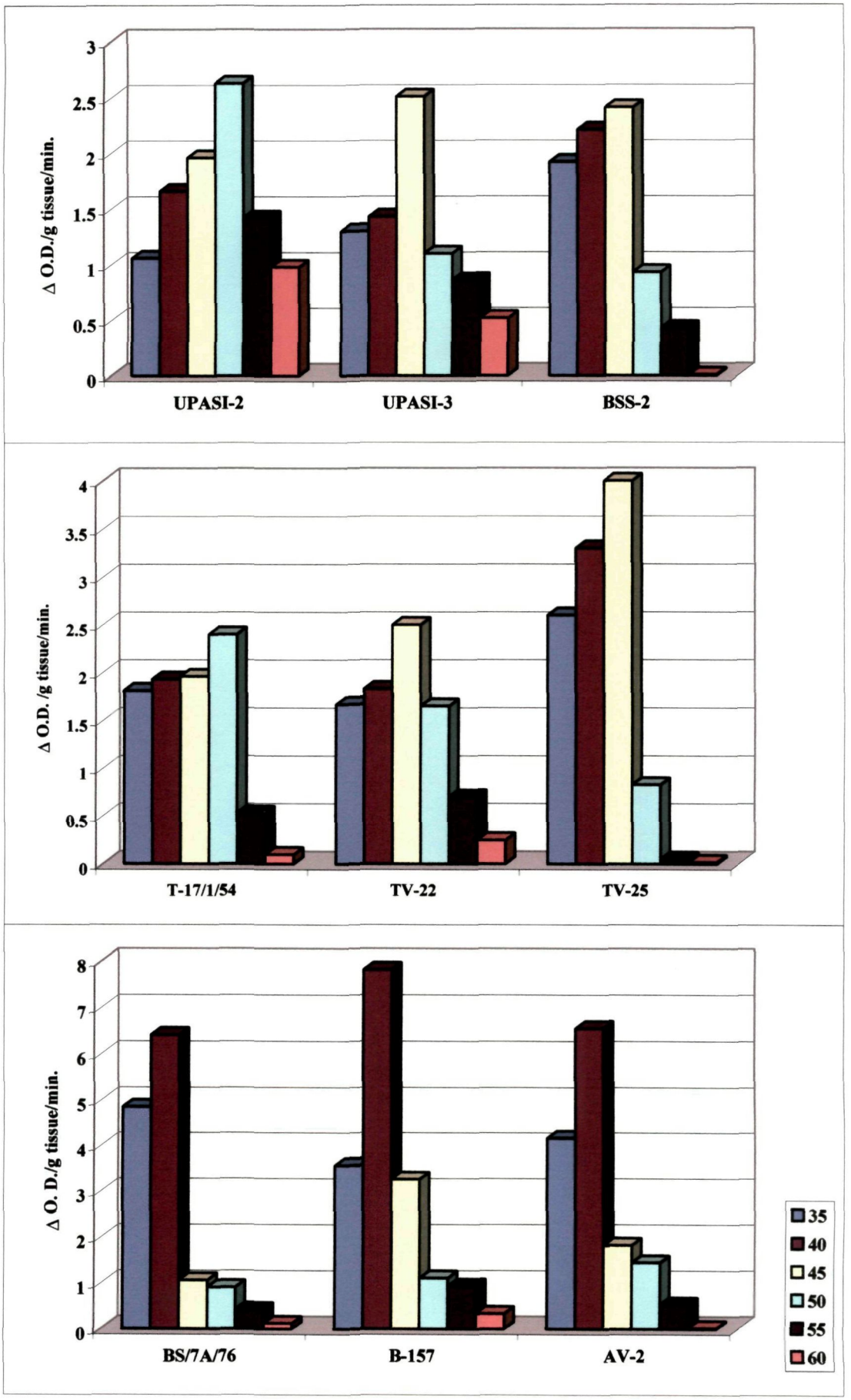


Fig.5

Table8: Effect of different temperatures on PPO activity in tea leaves

Varieties	PPO Activity (Δ O.D. /g tissue /min) Temperatures (^{0} C)					
	Control	40	45	50	55	60
UP-2	1.06±0.9	1.63±.13	1.96±.11	2.66±.22	1.42±.09	0.98±.01
UP-3	1.30±1.0	1.43±.04	2.51±.13	1.10±.21	0.86±.12	0.52±.10
UP-8	1.33±1.2	2.65±.01	2.86±.02	1.23±.17	0.78±.23	0.76±.15
UP-9	1.45±1.1	1.80±1.9	2.51±.18	2.00±.13	1.50±.12	0.98±.14
UP-26	1.33±0.7	1.79±.04	2.52±.13	0.76±.03	0.68±.78	0.51±.14
BSS-1	1.15±0.5	1.18±.09	2.25±.08	0.81±.04	0.21±.01	0.02±.18
BSS-2	1.92±1.1	2.21±.12	2.41±.04	0.93±.41	0.43±.03	0.01±.03
T-17/1/54	1.81±.02	1.93±.14	1.96±.14	2.40±.12	0.53±.12	0.10±.01
TV-9	1.03±.04	1.93±.13	2.55±.04	0.98±.78	0.13±.21	0.02±.11
TV-18	1.16±.07	1.35±.12	1.96±.12	2.32±.05	0.03±.08	0.01±.23
TV-22	1.67±.16	1.83±.03	2.50±.02	1.65±.02	0.70±.12	0.25±.14
TV-23	1.41±.14	1.65±.09	2.06±.12	0.83±.01	031±.07	0.12±.13
TV-25	2.60±.09	3.30±.12	4.00±.14	0.83±.01	0.05±.45	0.02±.70
TV-26	1.56±.44	2.01±.17	2.31±.06	1.38±.08	0.94±.34	0.52±.35
BS/7A/76	4.85±.21	6.41±1.1	1.06±.02	0.91±.08	0.42±.87	0.12±.13
T-78	3.71±13	7.57±.09	1.18±.11	0.76±.15	0.54±.13	0.23±.13
B-157	3.56±17	7.82±0.2	3.26±.13	1.11±.21	0.93±.07	0.34±.10
T-135	3.31±.04	5.29±.08	1.68±.17	1.70±.12	0.08±.34	0.03±.01
HV-39	3.36±.03	5.96±.03	2.07±.19	1.20±.10	0.02±.62	0.04±.02
AV-2	4.16±.11	6.55±.01	1.83±1.1	1.45±.02	0.53±.41	0.02±.01

Table 8a: Analysis of variance of data presented in Table 8

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	5	132.347	26.469	21.4795	0.69647
Varieties	19	31.088	1.636	1.3278	
Error	95	117.069	1.232		
Total	119	280.504			

After the optimum temperature, there was a decline in activity. Activity at 50^0C was significantly lower than that at all other temperatures.

Table 9: Effect of different temperatures on PPO activity of young tea seedlings

Varieties	PPO activity (Δ O.D. /g tissue /min.) Temperature (^{0}C)			
	Control	40	45	50
T-17/1/54	0.66 ±.01	1.15±.78	1.16±.018	0.60±.23
TV-18	1.26±.19	1.98±.38	1.16±.10	0.38±.19
TV-23	4.00±.02	4.86±.04	4.20±.18	2.00±.05
TV-25	3.36±.19	4.72±.05	4.20±1.0	2.20±.14
TV-26	1.46±.14	1.80±.04	1.61±.14	0.33±.02
T-78	3.78±.90	4.50±1.3	0.86±.18	0.13±.17
HV-39	4.80±.14	7.93±.03	5.10±.04	1.80±.14
UPASI-3	1.54±.10	7.64±1.2	2.54±.17	1.82±.09
UPASI-9	2.17±.03	3.48±.04	6.45±.23	0.64±1.8
BSS-2	5.80±.10	1.31±.02	1.10±.09	0.83±.09

Table 9a: Analysis of variance of data presented in Table 9

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	3	37.842	12.614	5.2822	1.63996
Varieties	9	60.928	6.770	2.8349	
Error	27	64.477	2.388		
Total	39	163.247			

4.2.1.2. Drought

PPO activity in the water stressed plants showed an initial increase after 4 days of treatment in all the tested varieties. Results are presented in Table 10 and Fig.6. Further water stress however led to significant decrease in activity .The activity after 4 and 12 days of drought were significantly different from that of control.

Table 10 : Effect of water stress on PPO activity in young tea seedlings

Tea variety	Polyphenol oxidase (Δ O.D. /g tissue / min.x 100) Days of water stress			
	Control	4	8	12
TV-18	1.80 ± 1.0	6.36±.03	1.76±.01	0.70±.02
TV-26	1.40 ± .04	6.21±.19	1.30±18	1.30±.19
UPASI-3	1.68 ±.98	3.36±.02	1.84±.11	0.68±.40
UPASI-26	2.08 ± 1.1	4.95±.17	1.51±.18	0.16±1.0
T-78	4.19 ±1.0	5.61±.02	2.55±.06	0.16±.07
HV-39	4.22 ± .05	4.55±.11	2.11±.18	0.63±..24

Table 10a: Analysis of variance of data presented in Table 10

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	3	66.972	22.324	26.7738	1.31271
Varieties	5	4.112	0.822	0.9863	
Error	15	12.507	0.834		
Total	23	83.591			

4.2.2. Anthropogenic

4.2.2.1. Fungicide

There was an increase in PPO activity in the leaves following first and second spray with hexaconazole which then decreased after the third spray, but the decrease was not statistically significant (Table 11).

Table 11: Effect of fungicide on PPO activity of young tea seedlings

Tea varieties	PPO activity (Δ O.D. /g tissue /min.)			
	Control	1st spray	2nd spray	3rd spray
TV-18	1.19 ± 0.61	2.70 ± 0.3	5.5 ± 0.92	1.22 ± 1.08
TV-26	1.28 ± 1.20	3.36 ± 0.7	2.20 ± 1.1	0.20 ± 0.15

C.D.(5%) for treatment mean effects = 4.93901

Effect of abiotic stress on PPO activity in tea seedlings

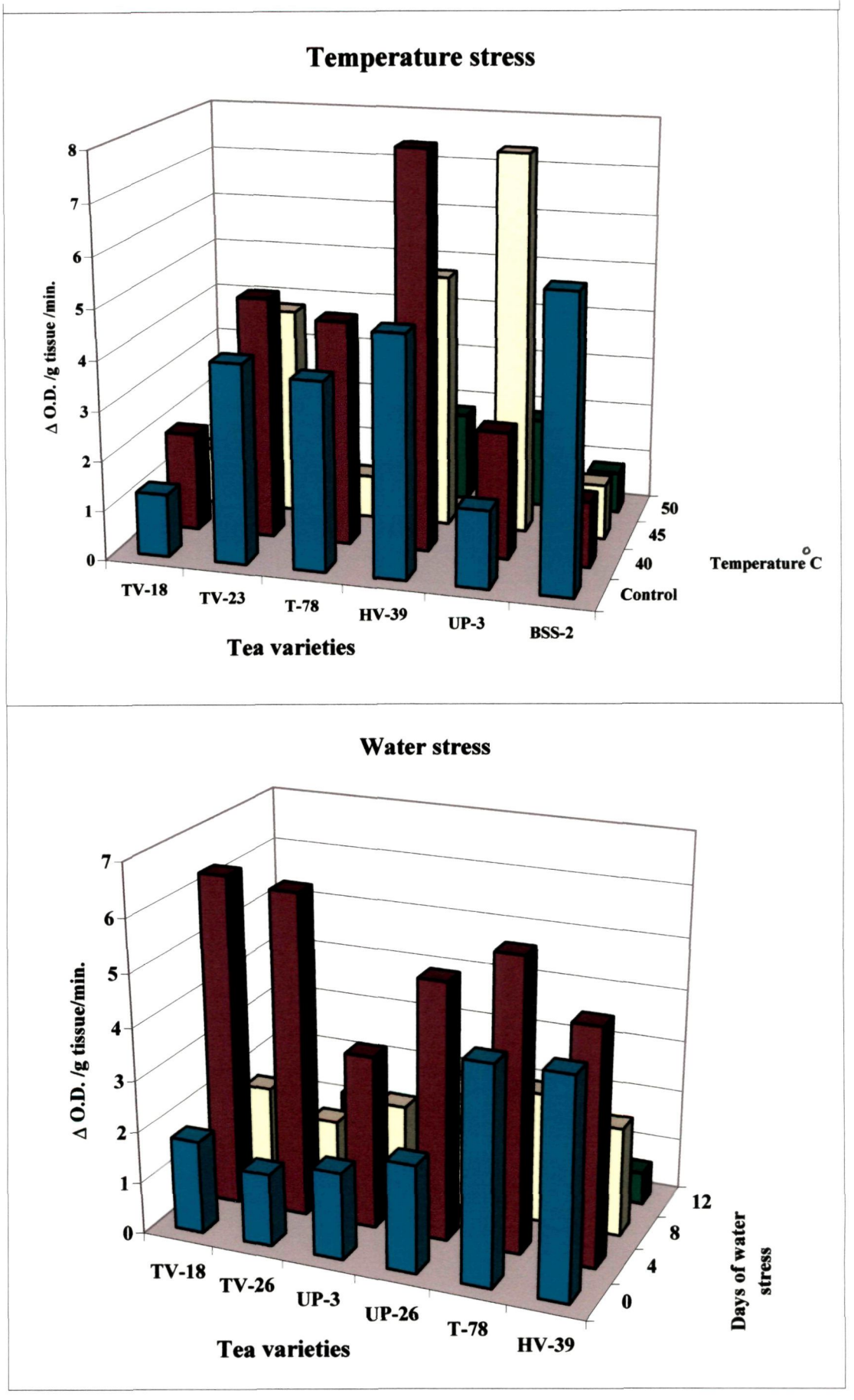


Fig.6

4.2.2.2. Insecticide:

In case of spray with monocil also similar results were obtained with PPO activity registering increase following first and second sprays and then show a decline (Table 12).

Table 12: Effect of insecticide on PPO activity on young tea plants

Tea varieties	PPO activity (Δ O.D. /g tissue /min.)			
	Control	1st spray	2nd spray	3rd spray
TV-18	1.19 ± 0.2	2.19 ± 1.2	5.90 ± 0.6	1.31 ± 0.8
TV-26	1.28 ± 0.2	1.32 ± 0.9	4.95 ± 1.1	0.20 ± 1.5

C.D.(5%)for treatment mean effects = 0.88743

4.2.3. Biotic stress

PPO activity was higher in the infected leaves in comparison to the healthy ones in all cases, but the overall increase was not statistically significant (Table 13.)

Table 13:Effect of blister blight infection on PAL activity of tea plants

Tea gardens	PPO activity (Δ O.D. /g tissue /min.)	
	Healthy leaves	Infected leaves
MarH TG	3.80 ±1.60	6.41 ± 0.98
Dil TG	5.11 ± 0.98	5.46 ± 1.22
Sin TG	4.84 ± 1.32	5.42 ± 1.01
Cas TG	5.8 0± 1.40	6.80 ± 1.10
Mok TG	4.70 ± 1.20	6.03 ± 1.32
Mar TG	4.10 ± 1.32	4.60 ± 1.42
Has TG	1.65 ±0.04	1.06 ± 1.61

C.D.(5%) for treatment mean effects = 1.1095

PPO activity in general showed an increase following the various stresses. Continuation of the stress for longer extents led to decline in activiity.

4.3. Effect of different stresses on peroxidase activity of tea leaves

Peroxidase was extracted from tea leaves and its activity was assayed. Since there are previous reports on the presence of different isozymes of peroxidase, in the present study the isozyme pattern analysis was also done following different stresses.

4.3.1. Peroxidase activity

Activity was assayed as increase in absorbance when O-dianisidine was oxidised by the oxygen released from H_2O_2, which was oxidised by the enzyme.

4.3.1.1. Abiotic stress

4.3.1.1.1. Elevated temperature

Elevated temperature treatment up to 60^0C detached leaves was applied to the twenty varietiesand peroxidase activity was assayed. Results are presented in Table 14 and Fig.7.

Table 14: Effect of different temperatures on peroxidase activity in tea leaves

Varieties	Peroxidase activity (Δ O. D /g tissue /min.) Temperatures (°C)				
	Control	40	45	48	50
UP-2	09.4 ± 0.82	05.7±0.1	01.5±.12	0.14±.21	0.08±.22
UP-3	13.2 ± 0.1	10.6±0.20	1.2±1.80	0.78±0.13	0.12±1.10
UP-8	07.6 ± 0.12	04.8± 0.09	02.4±1.2	1.08±0.12	0.23±1.64
UP-9	09.9 ± 0.45	07.4± 0.13	02.4±0.9	0.84±0.12	0.12±0.23
UP-26	08.0 ± 0.18	03.0 ± .03	01.3±.1.09	0.82±0.12	0.12±0.15
BSS-1	06.4 ± 0.11	08.0 ± 0.12	08.0±1.21	01.6±0.11	01.8±0.73
BSS-2	06.4 ± 1.01	08.6 ± 0.12	07.6±0.98	03.2±0.25	01.6±0.17
T-17/1/54	07.2 ± 0.82	08.1± 0.14	07.5±0.07	01.8±0.14	00.8±1.03
TV-9	09.6 ± 0.81	09.0 ± 1.10	04.2±0.83	02.6±.74	01.2±0.63
TV-18	06.4 ±.0.12	09.0 ± 1.63	06.3±1.02	01.8±9.23	00.6±0.73
TV-22	09.6 ± 0.13	09.6 ± 0.11	03.3±1.18	02.6±0.45	00.8±0.62
TV-23	06.6 ± 0.14	07.5 ± 0.14	02.4±0.98	01.8±0.12	00.3±1.29
TV-25	07.4 ± 0.11	10.5 ± 0.14	04.2±1.24	01.8±0.33	00.8±1.03
TV-26	11.4 ± 0.09	05.4 ± 0.12	02.4±.0.86	1.16±0.21	0.05±0.43
BS/7A/76	08.6 ± 1.02	12.2 ± 0.16	04.0±1.56	01.4±0.23	01.2±0.53
T-78	05.2 ±.0.43	07.5 ± 0.08	02.9±0.84	01.4±0.52	01.6±0.52
B-157	06.0 ±.0.12	09.6 ± 0.15	04.1±1.64	02.2±0.63	01.8±1.29
T-135	08.4 ± 0.12	10.4 ± 0.89	04.0±3.54	01.6±1.28	01.8±0.62
HV-39	07.4 ± 0.16	09.0 ± 1.15	03.6±1.94	00.1±1.16	00.8±1.10
AV-2	09.4 ± 0.12	11.2 ± 1.18	02.6±1.61	01.6±0.74	0.02±1.28

Effect of elevated temperatures on PO activity in tea leaves

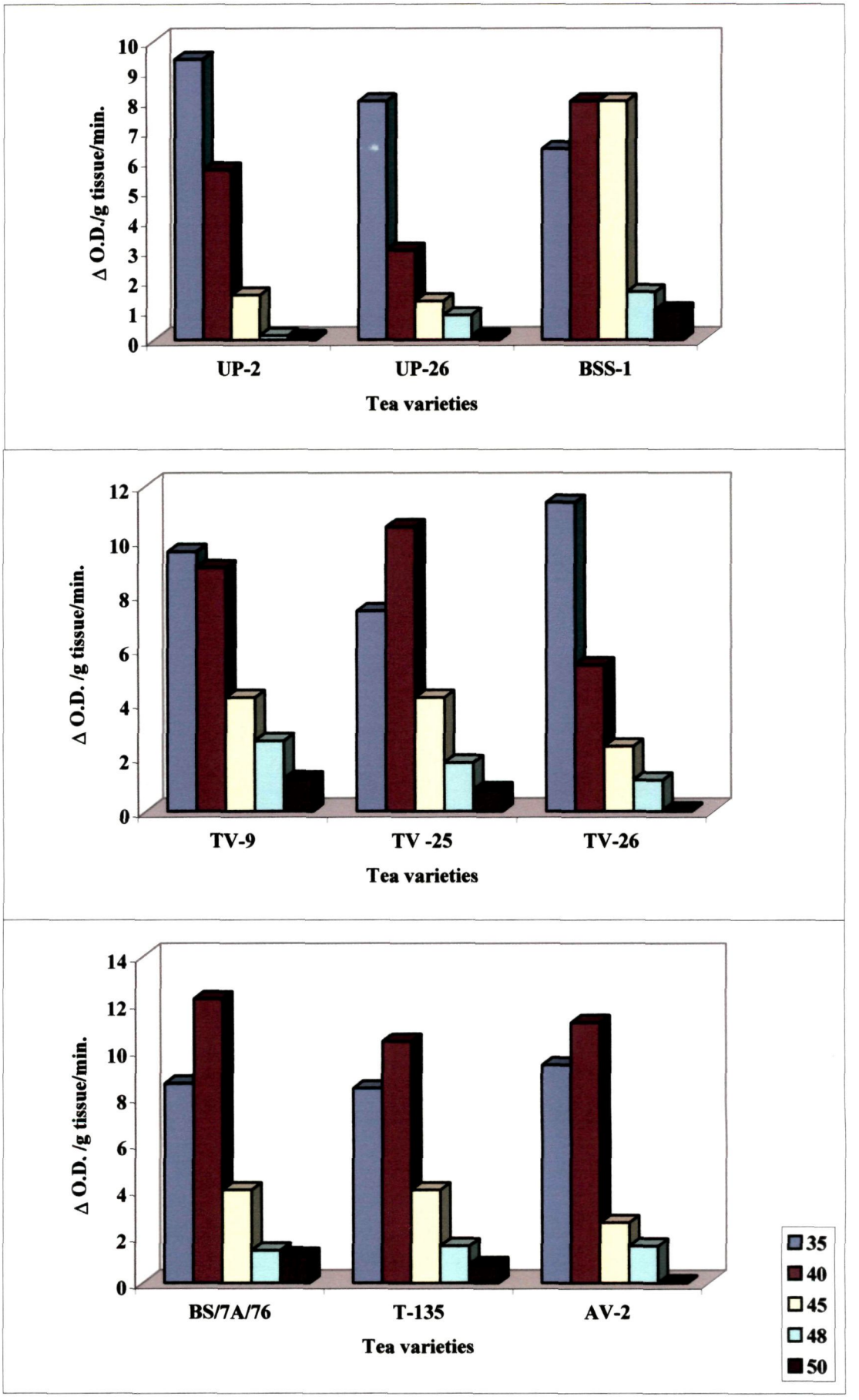


Fig.7

Table 14 a: Analysis of variance of of data presented in Table 14

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	4	1047.984	261.996	97.3734	1.18682
Varieties	19	68.076	3.583	1.3316	
Error	76	204.488	2.691		
Total	99	1320.547			

Activity was observed to decrease significantly after 40^0C and no activity could be detected after 50^0C. Even at 50^0C activity in most of the varieties was minimal.

In case of intact plants peroxidase activity showed a steady decline at all elevated temperatures, which was statistically significant (Table 15, Fig.8).

Table15. Effect of elevated temperatures on peroxidase activity in tea plants

Varieties	Peroxidase activity (Δ O. D. /g tissue /min.) Temperature (^{0}C)			
	Control	40	45	50
T-17/1/54	9.02±1.92	7.10±1.72	2.80±1.28	1.60±.92
TV-18	11.6±1.93	9.18±2.74	6.23±1.12	4.59±2.01
TV-23	14.2±1.72	13.93±1.20	3.59±1.93	1.62±1.83
TV-25	10.8±2.73	6.66±4.02	2.81±1.63	1.06±2.02
TV-26	11.9±3.52	10.2±2.02	6.20±3.02	3.86±2.00
T-78	10.1±1.92	5.98±1.93	4.08±2.84	1.16±1.68
HV-39	11.6±2.19	9.78±1.10	5.21±1.82	3.48±1.50
UPASI-3	8.53±1.83	7.60±1.29	2.42±2.83	0.02±1.22
UPASI-9	7.17±1.94	6.48±1.27	2.45±1.63	0.64±1.12
BSS-2	7.94±0.93	6.89±1.11	0.49±2.03	0.02±1.02

Table 15a: Analysis of variance of of data presented in Table 15

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	9	474.269	158.090	103.906	1.30901
Varieties	3	108.344	12.038	7.9123	
Error	27	41.079	1.521		
Total	39	623.692			

4.3.1.1.2. Drought

Peroxidase activity in leaves of plants subjected to water stress registered an increase. Activity was maximum after 4 days of water stress and then declined with further stress, in the Tocklai and Darjeeling varieties. However, in the UPASI varieties, even after 12 days of water stress the activity was higher than that of control (Table 16, Fig.8).

Table 16: Effect of water stress on peroxidase activity in young tea plants

Tea variety	Peroxidase activity (Δ O.D. /g tissue / min) Days of water stress			
	Control	4	8	12
TV-18	12.80±1.15	21.29±1.28	16.56±1.28	06.31±1.11
TV-26	10.12±1.28	10.93±1.54	08.80±1.11	03.83±1.02
UPASI-3	09.26±1.93	10.19±3.02	10.68±1.02	15.25±1.84
UPASI-26	08.58±1.68	11.29±2.65	12.58±1.18	13.57±2.03
T-78	10.00±2.02	16.75±2.04	08.80±1.03	02.85±4.02
HV-39	11.60±2.01	12.90±2.28	14.00±2.03	04.77±2.98

Table 16a: Analysis of variance of data presented in Table 16

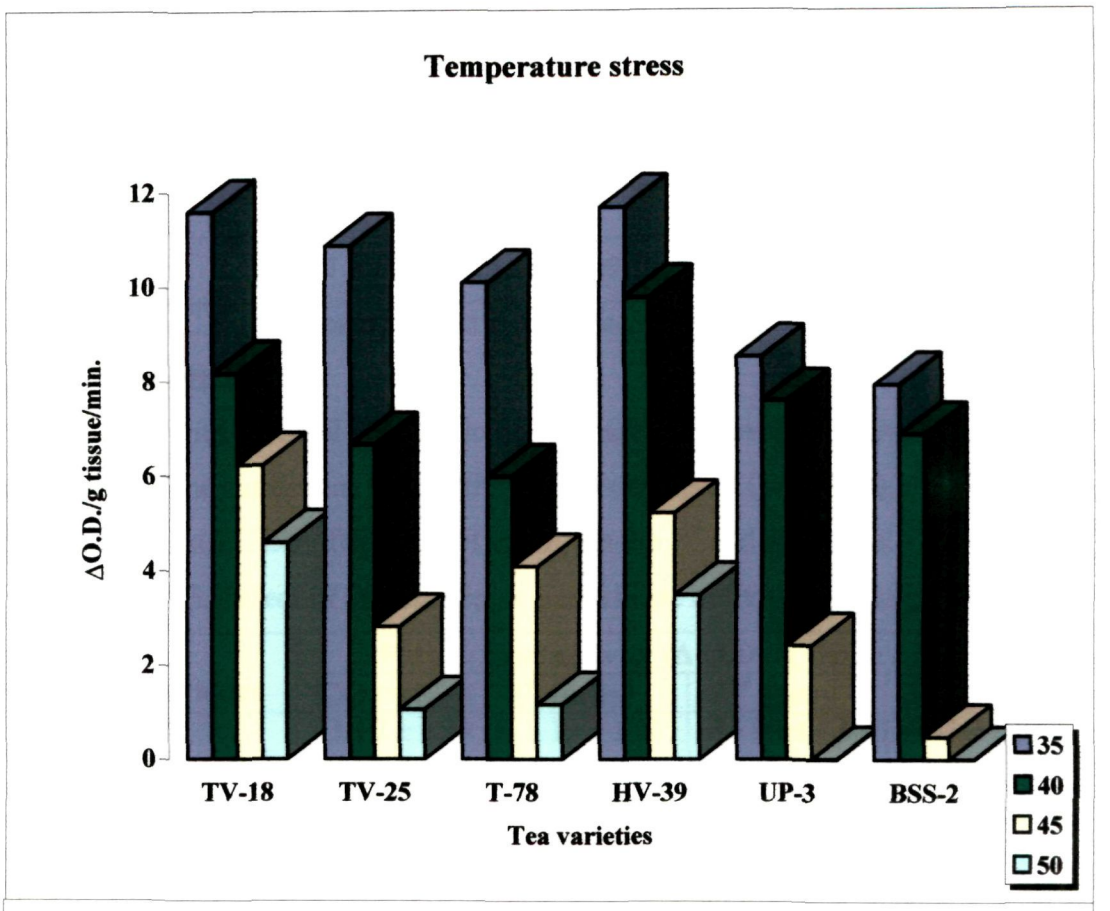
Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	5	120.127	40.042	2.7956	5.44075
Varieties	3	78.082	15.616	1.0903	
Error	15	214.848	14.323		
Total	23	413.057			

4.3.1.2. Anthropogenic

4.3.1.2.1. Fungicide

Increased activity of peroxidase was detected following spray with hexaconazole in both the varieties. Following second spray the activity was maximum which was almost double of that control (Table 17).

Effect of abiotic stress on PO activity in tea seedlings



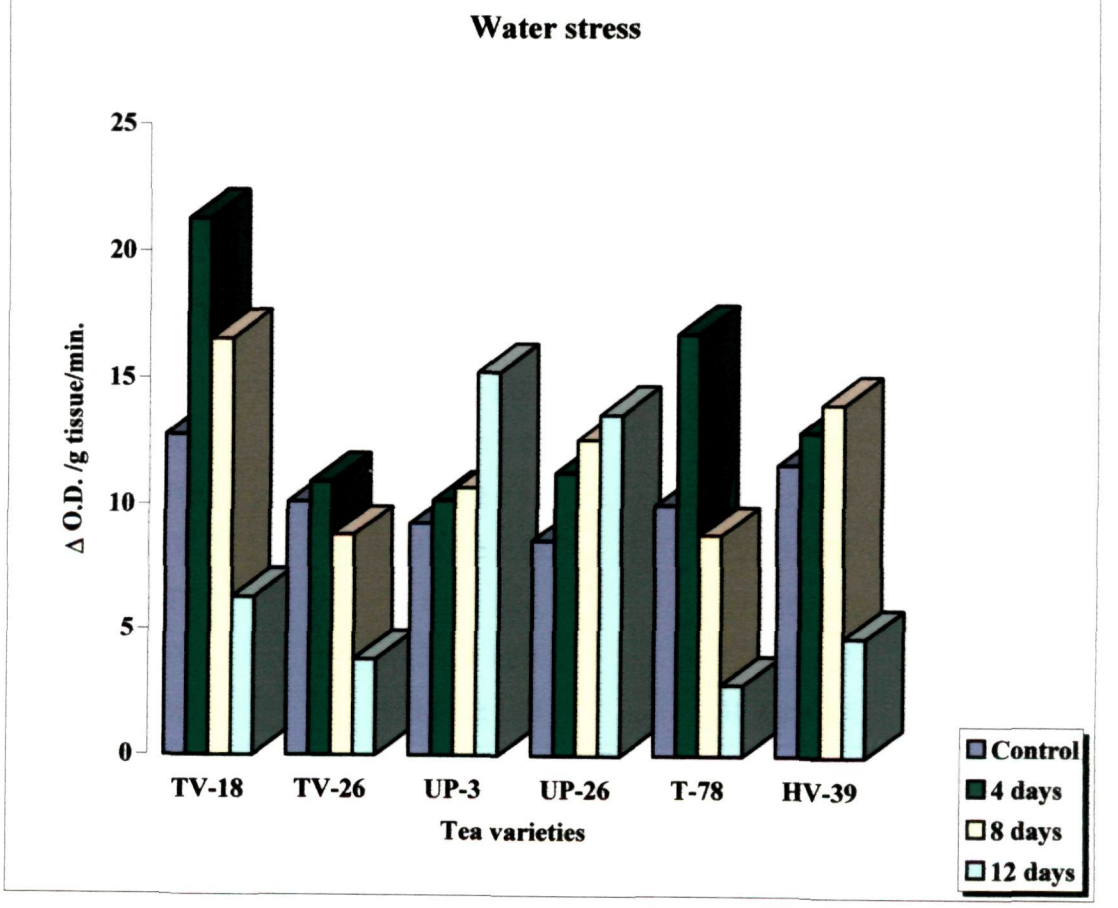


Fig.8

Table 17: Effect of fungicide on peroxidase activity of young tea plants

Tea varieties	Peroxidase activity (Δ O.D. /g tissue / min)			
	Control	1st spray	2nd spray	3rd spray
TV-18	11.92 ± 0.21	12.33 ± 1.2	20.90 ± 0.69	10.31 ± 0.08
TV-26	9.27 ± 1.17	10.32 ± 0.09	14.95 ± 1.10	07.20 ± 1.50

C.D.(5%) for treatment mean effects = 5.08641

4.3.1.2.2. Insecticide

Spraying with monocil also led to an increase in preoxidase activity in both the varieties with the maximum being after the second spray (Table 18).The difference in activity was significant from that of control in the 3rd and 4th sprays.

Table 18. Effect of insecticide on peroxidase activity of young tea plants

Tea varieties	Peroxidase activity (Δ O.D. /g tissue / min)			
	Control	1st spray	2nd spray	3rd spray
TV-18	11.92 ± 0.24	13.19 ± 1.20	15.90 ± 0.69	6.31 ± 0.08
TV-26	09.27 ± 0.74	12.32 ± 0.09	14.95 ± 0.81	4.20 ± 0.58

C.D.(5%) for treatment mean effects = 2.58693

4.3.1.3. Biotic

Infection by E. *vexans* led to a statistically significant increase in peroxidase activity in all cases, which was almost double to that of control. Artificial inoculation with spores also led to an increase in activity (Table 19).

Table 19: Effect of blister blight infection on peroxidase activity in tea plants

Tea gardens	Peroxidase activity (Δ O.D./g tissue /min)	
	Healthy leaves	Infected leaves
MarH TG	5.6 ± 1.10	12.8 ± 1.23
Dil TG	6.0 ± 1.82	12.0 ± 1.09
Sin TG	11.2 ± 0.09	15.2 ± 0.67
Cas TG	11.2 ± 0.86	14.0 ± 1.19
Mok TG	10.4 ± 1.18	15.2 ± 0.09
Mar TG	8.4 ± 1.18	17.6 ± 0.76
Has TG	3.6 ± 1.10	4.31 ± 1.12
Art.ino.	14.2 ± 1.11	18.1 ± 0.87

C.D.(5%) for treatment mean effects = 2.65030

Results of all the experiments on the effect of various stresses on preoxidase activity revealed that elevated temperature alone resulted in a decrease in the activity whereas all the other stresses induced greater activity. It seems probable therefore that the enzyme is unstable at high temperature and hence the activity decreases. The general response of plants to stress could be that of an increase in activity.

4.3.2. Isozymes of peroxidase

Presence of multiple isozymes of peroxidase in many plants including tea has been previously reported. Experiments were conducted to determine whether the different stresses induce the activity of different isozymes or whether the activity of any isozyme is lost. Analysis of isozymes was done on native PAGE gel and the bands were visualized using benzidine as hydrogen donor following the methods described under materials and methods. Detailed analysis was carried out in leaves subjected to abiotic and biotic stresses.

4.3.2.1. Abiotic stress

PAGE analysis of peroxidase isozyme was carried out in UP-3 for both elevated temperature and water stress. Results are presented below:

4.3.2.1.1. Elevated temperature

Presence of three isozyme bands of Rf values .375, .500 and .604 was visualized in control leaves. Peroxidase extracts from leaves subjected to elevated temperature also exhibited a more or less similar pattern to that of control leaves with three prominent bands. However at 40^0C or 50^0C an additional faint band could be visualized at Rm 0.77 (Table 20, Plate VIII).

Table20: Relative mobility of peroxidase isozymes of tea plants subjected to elevated temperatures

Isozyme nos.	Rm	Control	40^0C	45^0C	50^0C
1.	0.375	+	+	+	+
2.	0.500	+	+	+	+
3.	0.604	+	+	+	+
4.	0.770	-	+	-	+

4.3.2.1.2. Drought

In case of water stress also there was no difference in number of bands between control and stressed leaves as visualized on the gel. Three isozymes were evident in all the cases (Table 21, Plate VIII).

Table 21: Relative mobility values of peroxidase isozymes subjected to drought

Isozyme nos.	Rm	Control	4th day	8th day	12th day
1.	0.375	+	+	+	+
2.	0.500	+	+	+	-
3.	0.604	+	+	+	+

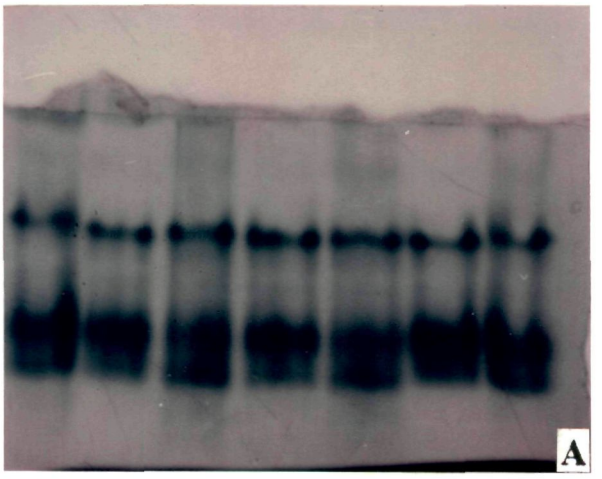
Analysis of isozymes revealed that no differences could be visualized in both the abiotic stresses i.e. elevated temperature and drought. However when activity of peroxidase enzymes was assayed significant changes were evident. Hence no correlation could be drawn in this case between activity and number or intensity of band.

4.3.2.2. Biotic stress

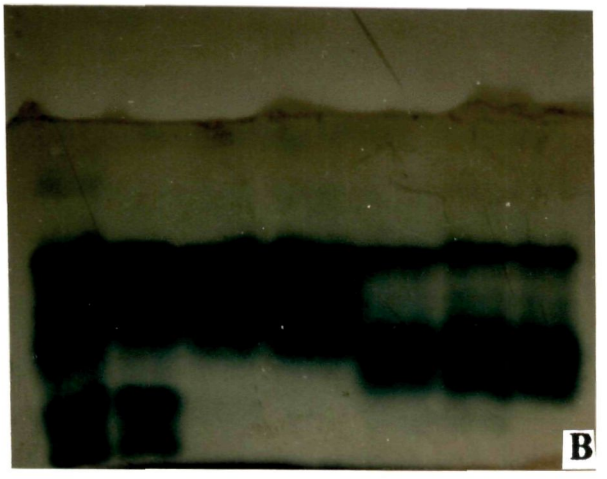
Peroxidase was extracted from healthy and blister blight infected leaves collected from Castleton tea gardens of Darjeeling area and were analysed by PAGE. Difference stages of infection i.e. young, mature and old blisters were used for analysis. It was observed that in the very young blister the band pattern was similar to that of control with the presence of three bands of Rm values .375, .5, and .604. In the mature and old blisters two additional bands of higher Rm values 0.77 and 0.89 were distinctly visible. In the old blisters a further faint bands of Rm 0.160 was also present (Table 22, Plate VIII).

Table 22: Relative mobility values of the isozymes of peroxidase from healthy and blister infected tea leaves

Isozyme Numbers	Relative Mobility	Healthy Leaves	Blister Infected Leaves		
			Stage 1	Stage 2	Stage 3
1.	0.160	+	-	-	+
2.	0.375	+	+	+	+
3.	0.500	+	+	+	-
4.	0.604	+	+	+	+
5.	0.770	-	-	+	+
6.	0.890	-	-	+	+



1 2 3 4 5 6 7



1 2 3 4 5 6 7

Plate VIII (figs.A & B): Peroxidase isozyme analysis of abiotic and biotic stressed tea plants(UP-3); A (Lanes 1-7) Temperature stress:1&7-50^{0}C, 2&6-45^{0}C, 3&5-40^{0}C, 4-Control; B - Lanes 1-4 : Blister infection & 5-7: Drought; 1-Old,2-Mature,3-Young &4-Healthy; 5- 4 days, 6-8 days & 7- 12 days drought

Thus blister infection seemed induce the activity of at least two new isozymes not detected in any of the other cases.

4.4.Changes in chlorophyll content of tea leaves following different stresses

Chlorophyll is the most important plant pigment since it controls the photosynthesis process of a plant and thereby determines the productivity of a plant. Any change in the chlorophyll content due to environmental conditions would thus alter the photosynthetic efficiency of the plant. Keeping this in mind, in the present study changes in chlorophyll due to various stresses have been determined.

4.4.1. Abiotic stress

4.4.1.1. Elevated temperature stress

Total chlorophyll, chl a and chl b contents of leaves of young tea seedlings subjected to elevated temperatures were determined. Results revealed that chlorophyll content decreased significantly at higher temperatures, particularly from 45^0C onwards (Table 23). All the differences among the treatments were statistically significant.

Among the different varieties chlorophyll content varied from around 1.3 mg to 1.7 mg per g fresh weight of tissue. Most significant effect of temperature on chlorophyll content was evident in the Darjeeling varieties, where the reduction at 50^0C was approximately 10 fold. While in the UPASI varieties there was a 50% reduction (Fig. 9). Similar observations were also recorded in case of chl a and chl b.

4.4.1.2. Drought

In case of water stress also reduction in chlorophyll content was recorded. Initially after 4 days of drought there was only marginal decrease in chlorophyll content. After 8 days and 12 days of water stress however significant reduction was apparent. UPASI-3 and UPASI-26 recorded the least (<50%) reduction (Table 24, Fig.10).

Table 23: Effect of different temperatures on chlorophyll content of young tea plants

Tea varieties	Chlorophyll content (mg/g tissue) Control	40	45	50
T-17				
Total Chl	1.316±.10	1.013±.98	0.415±.34	0.223±.10
Chl a	0.936±.11	0.771±.29	0.296±.02	0.106±.92
Chl b	0.381±.11	0.500±.11	0.118±.02	0.117±.12
TV-18				
Total Chl	1.471±.19	1.149±.19	0.613±.02	0.312±.80
Chl a	1.097±.12	0.865±.25	0.411±.23	0.112±.20
Chl b	0.374±.21	0.284±.11	0.202±.344	0.201±.25
TV-26				
Total Chl	1.268±.01	1.102±.90	0.764±1.92	0.396±.78
Chl a	0.925±.03	0.781±.32	0.506±.03	0.208±.34
Chl b	0.343±.04	0.328±.10	0.268±.02	0.188±.88
T-78				
Total Chl	1.438±.11	0.992±.03	0.378±.83	0.163±.37
Chl a	0.958±.13	0.582±.11	0.157±.92	0.055±.26
Chl b	0.481±.04	0.410±.02	0.221±.82	0.108±.92
HV-39				
Total Chl	1.325±.47	0.831±.37	0.416±1.0	0.141±.34
Chl a	1.011±.02	0.608±.17	0.236±0.2	0.071±.17
Chl b	0.315±.03	0.223±.02	0.180±.13	0.070±.16
UPASI-3				
Total Chl	1.680±.49	1.361±.11	1.116±.28	0.763±.26
Chl a	1.121±.28	1.011±.02	0.771±.93	0.591±.92
Chl b	0.469±.08	0.350±.34	0.345±.56	0.171±.23
UPASI-9				
Total Chl	1.716±.27	1.513±.13	1.212±.38	0.863±.18
Chl a	1.308±.02	1.118±.02	0.786±.20	0.560±.12
Chl b	0.406±.02	0.395±.19	0.321±.01	0.303±.13
BSS-2				
Total Chl	1.613±.02	1.489±.23	1.102±.72	0.865±.01
Chl a	1.201±.11	1.186±.02	0.751±.73	0.544±.03
Chl b	0.412±.03	0.403±.03	0.351±.93	0.316±.28

Table 23a: Analysis of variance of data presented in Table 23

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	3	5.605	1.868	182.014	0.10752
Varieties	9	2.201	0.245	23.8253	
Error	27	0.277	0.010		
Total	39	8.083			

Effect of elevated temperatures on chlorophyll content of tea seedlings

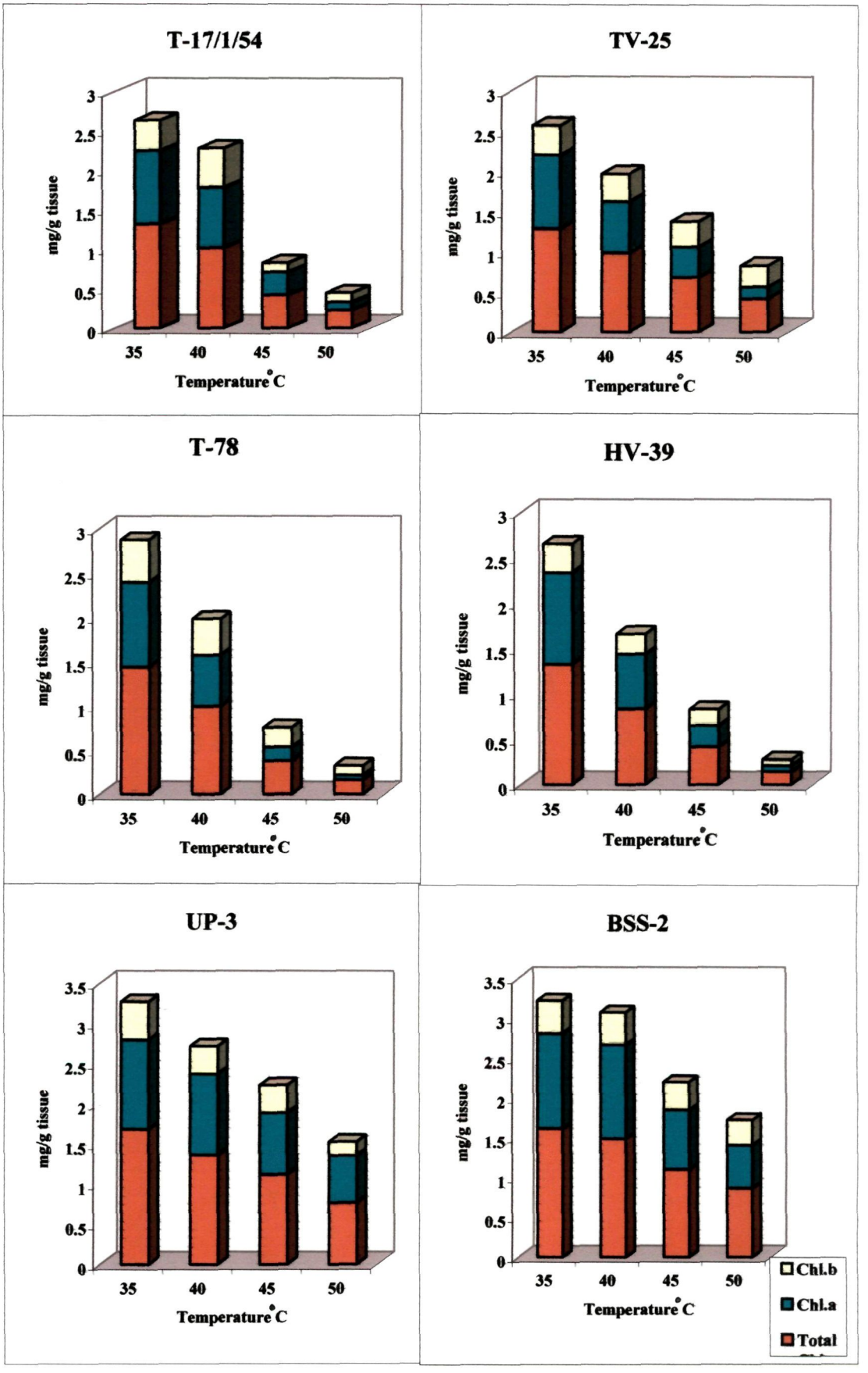


Fig.9

Table 24: Effect of water stress on chlorophyll content of young tea seedlings

Tea Variety	Chlorophyll content (mg/g tissue) Days of water stress			
	Control	4	8	12
TV-18				
Total Chl	1.261±1.1	1.241±1.09	0.415±1.20	0.223±.29
Chl a	0.987±1.02	0.731±.03	0.296±1.22	0.106±.21
Chl b	0.283±.98	0.500±.02	0.118±1.22	0.117±.22
TV-26				
Total Chl	1.027±1.67	0.937±1.12	0.362±.02	0.1678±.44
Chl a	0.723±1.82	0.589±1.16	0.129±.13	0.102±.54
Chl b	0.324±2.9	0.348±1.19	0.114±.02	0.066±.87
UPASI-3				
Total Chl	1.469±1.9	1.460±1.09	1.343±1.19	0.881±1.2
Chl a	0.961±1.92	0.945±0.93	0.890±1.20	0.669±2.12
Chl b	0.508±2.02	0.515±0.94	0.452±1.22	0.212±1.82
UPASI-26				
Total Chl	1.579±.03	1.570±1.98	1.438±1.11	0.981±1.2
Chl a	0.961±1.09	0.968±.03	0.890±1.10	0.669±1.1
Chl b	0.618±0.33	0.602±.04	0.548±1.1	0.302±1.3
T-78				
Total Chl	1.118±1.28	1.030±1.92	1.069±.96	0.108±.28
Chl a	0.765±1.23	0.635±1.27	0.766±.94	0.071±.62
Chl b	0.353±1.92	0.369±1.19	0.302±.82	0.039±.82
HV-39				
Total Chl	0.653±.04	0.652±2.01	0.298±0.22	0.089±.03
Chl a	0.466±.02	0.420±1.09	0.176±1.10	0.048±.02
Chl b	0.186±.02	0.233±1.09	0.122±1.1	0.041±.02

Table 24a: Analysis of varience of data presented in Table 24

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	3	2.342	0.781	24.1380	0.25856
Varieties	5	2.857	0.571	17.6658	
Error	15	0.485	0.032		
Total	23	5.685			

Effect of water stress on chlorophyll content of tea seedlings

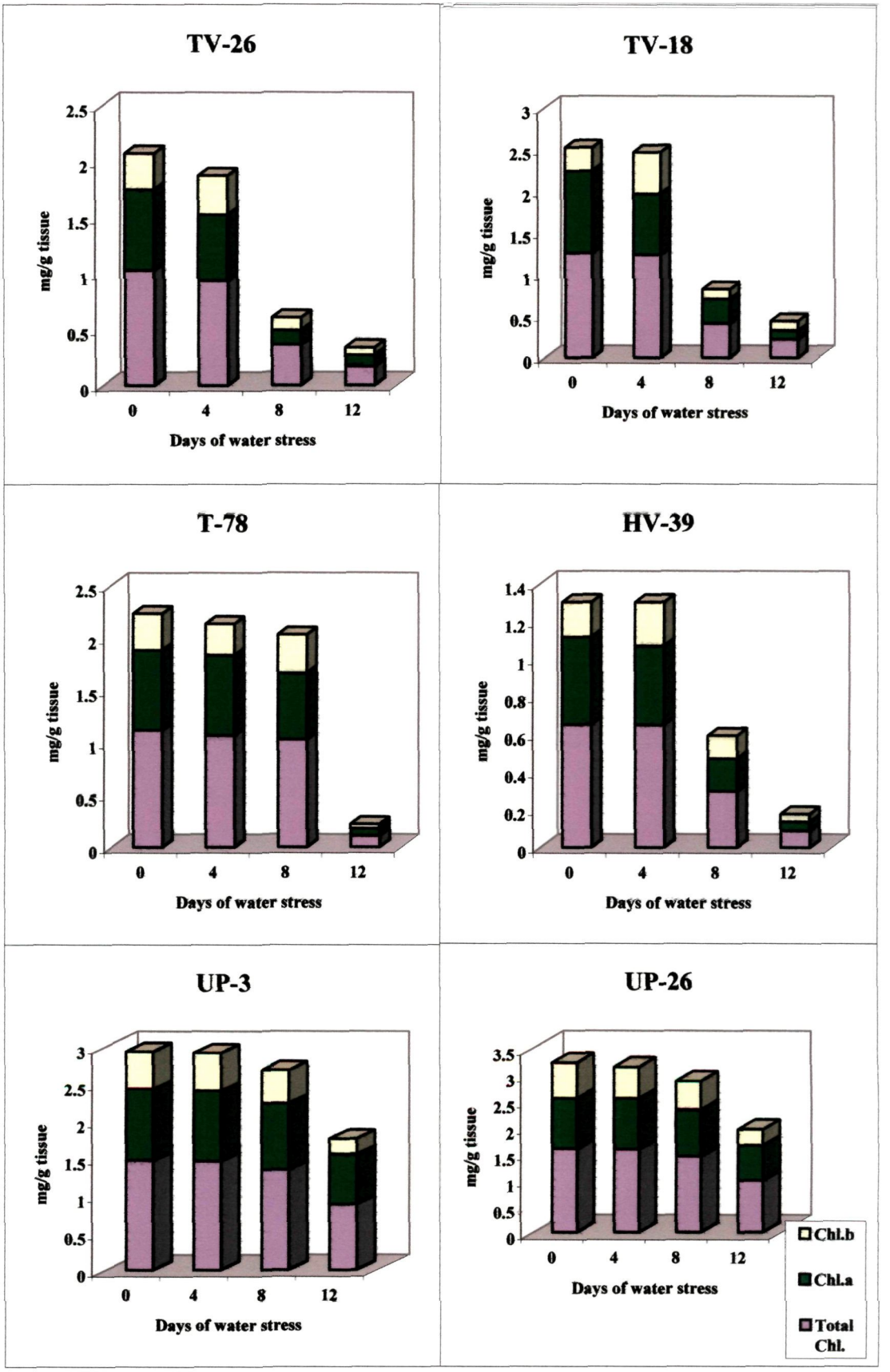


Fig.10

4.4.2. Anthropogenic stress

4.4.2.1. Fungicide

The first spray of hexaconazole did not significantly affect the chlorophyll content. Decrease in total chlorophyll, chl a and chl b were observed after 2nd and 3rd spray within the minimum being after the 3rd spray (Table 25, Fig.11). The difference in chlorophyll content after 3rd spray was significantly different from that of all other treatments.

Table 25: Effect of Hexaconazle on chlorophyll content of tea seedlings

Tea varieties	Chlorophyll content (mg/g tissue)			
	Control	1st spray	2nd spray	3rd spray
TV -18				
Total Chl	1.268±.02	1.214±.13	0.916±.14	0.340±.21
Chl a	0.986±.22	0.961±.02	0.746±.16	0.247±.24
Chl b	0.282±.11	0.250±.18	0.170±.18	0.093±.11
TV -26				
Total Chl	1.096±.23	1.068±.24	0.878±.11	0.501±.27
Chl a	0.723±.21	0.720±.21	0.591±.31	0.389±.10
Chl b	0.373±41	0.348±.26	0.287±.30	0.112±.12

C.D.(5%) for treatment mean effects = 0.44640

4.4.2.2. Insecticide

Spraying of plants with monocil affected chlorophyll content to a certain degree. Significant decrease in chlorophyll content was evident after the 3rd spray (Table 26, Fig.11).

Table 26: Effect of Monocil on chlorophyll content of young tea plants

Tea varieties	Chlorophyll content (mg/g tissue)			
	control	1st spray	2nd spray	3rd spray
TV -18				
Total Chl	1.268±.01	1.264±.26	1.018±.29	0.631±.28
Chl a	0.986±.12	0.971±.42	0.821±.25	0.482±.22
Chl b	0.282±.12	0.273±.33	0.197±.11	0.149±25
TV -26				
Total Chl	1.096±.12	1.006±1.0	0.977±.18	0.686±.28
Chl a	0.723±.16	0.707±.62	0.692±.13	0.419±.31
Chl b	0.373±.12	0.352±.66	0.285±.12	0.267±.23

C.D.(5%) for treatment mean effects = 0.40853

4.4.3. Biotic stress

Blister blight infection reduced the total chlorophyll content significantly in all the tested cases. Chlorophyll a content was found to decrease more significantly than chlorophyll b (Table 27, Fig. 11).

Table 27. Effect of blister blight infection on chlorophyll content of tea plants

Tea gardens		Chlorophyll content (mg/g tissue) Healthy leaves	Infected leaves
MarH TG	Total Chl	0.903±1.9	0.746±.42
	Chl a	0.551±1.23	0.441±.94
	Chl b	0.352±1.2	0.305±.93
Dil TG	Total Chl	0.929±1.92	0.659±1.19
	Chl a	0.581±1.02	0.408±1.29
	Chl b	0.348±1.1	0.251±1.27
Sin TG	Total Chl	0.987±2.03	0.720±.93
	Chl a	0.610±1.92	0.424±1.10
	Chl b	0.337±2.26	0.206±1.92
Cas TG	Total Chl	0.862±1.68	0.675±2.01
	Chl a	0.641±1.72	0.470±2.03
	Chl b	0.225±1.52	0.205±2.1
Mok TG	Total Chl	0.925±.98	0.762±3.02
	Chl a	0.719±.70	0.440±2.84
	Chl b	0.206±.62	0.225±2.96
Mar TG	Total Chl	0.880±1.16	0.596±1.20
	Chl a	0.578±.92	0.416±1.93
	Chl b	0.312±.80	0.180±1.82
Has TG	Total Chl	1.110±1.21	0.406±.93
	Chl a	0.612±1.92	0.215±.36
	Chl b	0.498±1.56	0.191±.98

C.D.(5%) for treatment mean effects = 0.21331

Effect of anthropogenic and biotic stress on chlorophyll content of tea seedlings

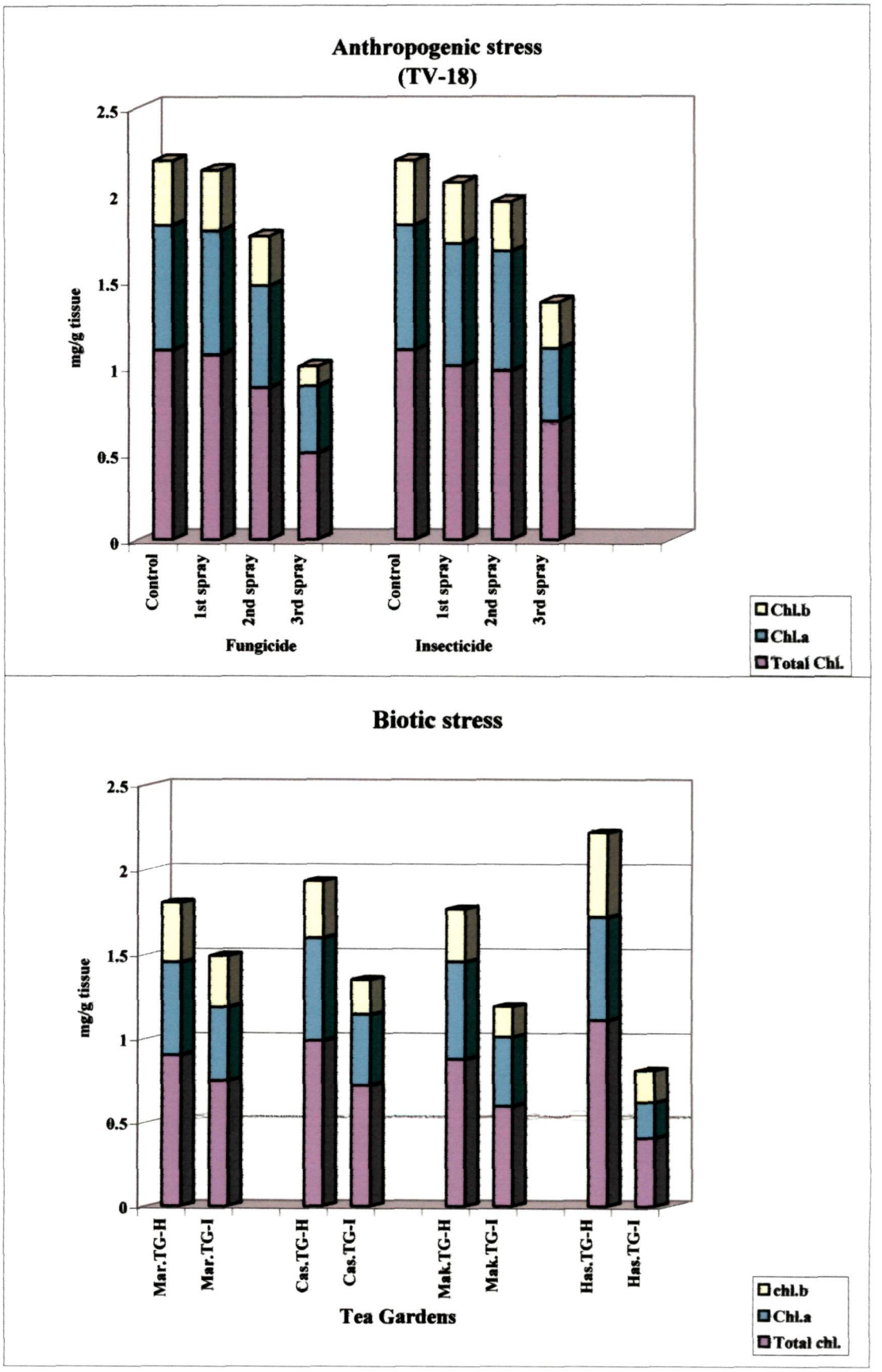


Fig.11

4.5. Effect of different of stresses on protein content of tea leaves

Changes in protein metabolism is the most important and immediate manifestation of stress response in plants. Stress can induce changes in protein accumulation as well as in the types of protein being synthesized. Effect of various stresses on both the protein content as well as the protein pattern of tea leaves were determined in the present study following standard methods already described. Results of this study have been presented below.

4.5.1. Protein content

Protein content was estimated in different varieties subjected to the various stresses following the method described in materials and methods.

4.5.1.1. Abiotic stress

4.5.1.1.1. Elevated temperature

Detached leaves of twenty varieties were subjected to elevated temperatures up to 60^{0}C and then protein content was estimated. Results (Table 28 and Fig. 12)

Table 28: Effect of different temperatures on protein content in tea leaves.

Varieties	Protein content (mg /g tissue) Temperatures (^{0} C)					
	Control	40	45	50	55	60
UP-2	30.08±1.0	31.57±1.9	30.75±.23	20.62±1.8	17.42±1.1	10.34±1.1
UP-3	31.60±.03	35.76±1.2	32.65±.11	21.1±1.2	10.86±1.3	10.43±.01
UP-8	33.75±1.1	32.08±.02	30.81±.32	20.75±.23	13.61±1.2	9.42±.02
UP-9	40.51±2.9	41.66±.68	40.50±.11	31.23±.24	16.1±1.9	13.39±1.2
UP-26	41.14±.96	40.27±.32	32.84±1.0	21.27±.21	11.1±.13	8.21 ±.03
BSS-1	30.52±.02	33.92±.21	32.07±.02	21.57±1.2	10.90±.72	10.72±.03
BSS-2	34.07±1.1	31.95±.01	31.76±.01	21.29±1.4	11.5±.24	9.50±.02
T-17	35.32±.36	35.17±.12	33.13±.14	22.10±1.4	21.04±1.9	10.89±.05
TV-9	32.75±1.1	32.65±.12	30.8±.12	20.80±1.9	20.6±.92	10.4±.04
TV-18	33.60±1.0	33.31±.46	31.35±.02	21.08±1.2	20.98±.18	10.54±.92
TV-22	32.95±.20	31.78±.03	30.96±.22	20.82±1.2	20.70±.24	10.62±.72
TV-23	35.25±.23	35.17±1.0	33.95±.93	21.68±1.1	21.14±.22	10.91±.23
TV-25	32.53±.12	31.95±.18	31.57±.63	20.90±.03	20.82±.21	10.70±.20
TV-26	34.51±.16	34.16±.17	33.77±.02	21.60±.52	20.77±1.9	10.68±.21
BS/7A/76	33.38±.11	32.98±.12	32.45±1.6	21.30±.12	20.82±.02	10.31±.10
T-78	35.25±.02	34.15±.32	23.15±.02	21.40±1.3	15.23±1.3	9.68±.03
B-157	33.30±.09	34.12±.02	23.73±1.7	22.30±1.2	12.27±1.0	10.92±.02
T-135	32.65±.98	31.26±1.9	20.92±1.9	20.68±.02	10.59±.12	8.40±.03
HV-39	33.75±.46	33.15±1.9	22.88±.16	22.01±1.0	11.05±.11	10.32±1.0
AV-2	34.17±.22	33.60±1.2	23.49±.02	21.50±.02	12.05±.01	10.36±.02

Effect of elevated temperatures on protein content of tea leaves

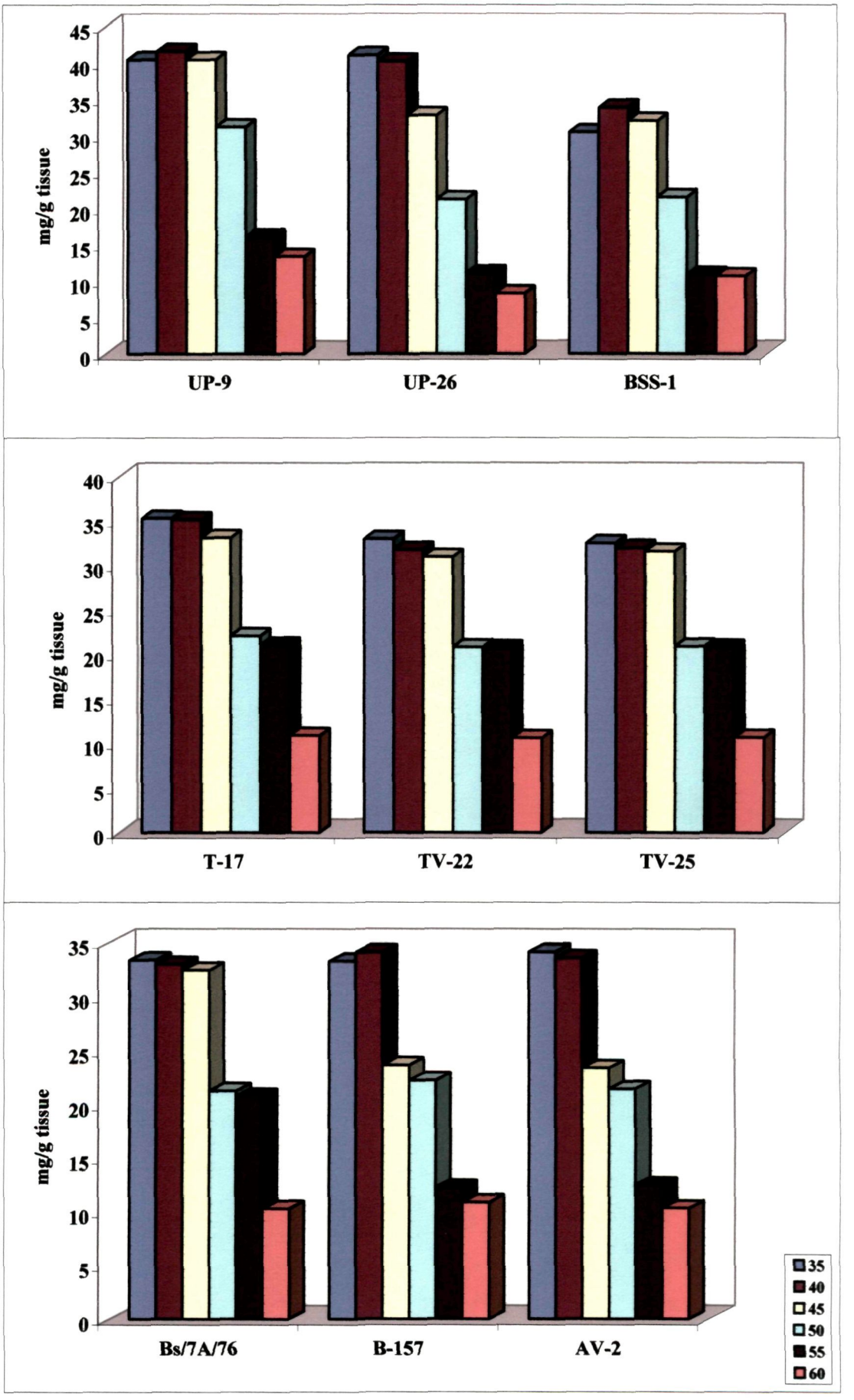


Fig.12

Table 28a: Analysis of varience of data presented in Table 28

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	5	9907.62	1981.52	258.796	1.73605
Varieties	19	494.971	26.051	3.4024	
Error	95	727.386	7.657		
Total	119	11129.98			

revealed a gradual decrease in protein content with increase in temperature. No significant difference was obtained at 40^0C, but further increase in temperatures led to significant differences in protein content. Differences among elevated temperatures were also significant.

When the whole plants were subjected to elevated temperature in some of the varieties there was an increase at 40^0C and then a decline. In the other varieties protein content decreased with all elevated temperatures (Table 29). There was a sitatistical difference in protein contents at 45 and 50^0C with those at control and 40^0C.

Table 29: Effect of different temperature on protein content of tea seedlings

Varieties	Protein content (mg/g tissue) Temperature (^{0}C)			
	Control	40	45	50
T-17/1/54	49.3±1.86	50.2±1.2	34.3±.08	30.8±2.01
TV-18	42.9±1.22	40.1±.72	36.0±.94	31.9±.3.02
TV-23	39.0±1.34	39.8±.27	20.6±1.0	22.3±1.52
TV-25	30.9±2.46	41.6±.28	31.7±1.2	28.8±1.21
TV-26	45.0±2.46	40.5±.92	40.0±1.5	39.0±1.1
T-78	35.3±1.12	36.8±1.8	31.6±.96	30.1±2.03
HV-39	40.0±.03	40.9±1.3	35.4±2.0	31.7±2.83
UPASI-3	56.0±.08	54.2±.03	52.6±2.61	41.2±2.23
UPASI-9	60.2±.48	58.0±1.11	52.8±3.02	40.0±3.04
BSS-2	59.8±2.0	5.82±1.12	51.0±4.5	46.0±2.1

Table 29a: Analysis of varience of data presented in Table 29.

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	3	1088.580	362.860	30.2602	3.67491
Varieties	9	2511.883	279.098	23.2750	
Error	27	323.766	11.991		
Total	39	3924.228			

4.5.1.1.2. Drought

Prolonged period of drought led to a decrease in protein content of all the tested varieties (Table 30). The decrease was highly significant in case of the Tocklai varieties (TV-18, TV-26) and Darjeeling varieties (T-78, HV-39). In UPASI varieties initial increase after 4 days of drought was observed after which there was a decline. However, the decrease was not significant even after 12 days (Fig. 13).

Table 30: Effect of water stress on protein content of young tea plants

Tea variety	Protein content (mg/g tissue) Days of water stress			
	Control	4	8	12
TV-18	32.40±4.47	38.05±2.03	31.50±4.0	20.55±2.03
TV-26	48.30±4.92	39.01±3.44	27.00±4.74	21.45±5.43
UPASI-3	54.16±5.01	46.18±3.8	33.20±6.92	32.18±4.73
UPASI-26	50.04±2.03	46.25±5.93	42.50±6.33	30.06±2.73
T-78	27.60±6.06	30.00±2.03	28.05±2.03	27.45±3.34
HV-39	32.35±5.53	27.45±3.20	27.45±3.38	21.75±2.02

Table 30a: Analysis of variance of data presented in Table 30

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	3	1303.896	434.632	20.5831	6.60608
Varieties	5	738.697	147.739	6.9966	
Error	15	316.739	21.116		
Total	23	2359.332			

Effect of abiotic stress on protein content of tea seedlings

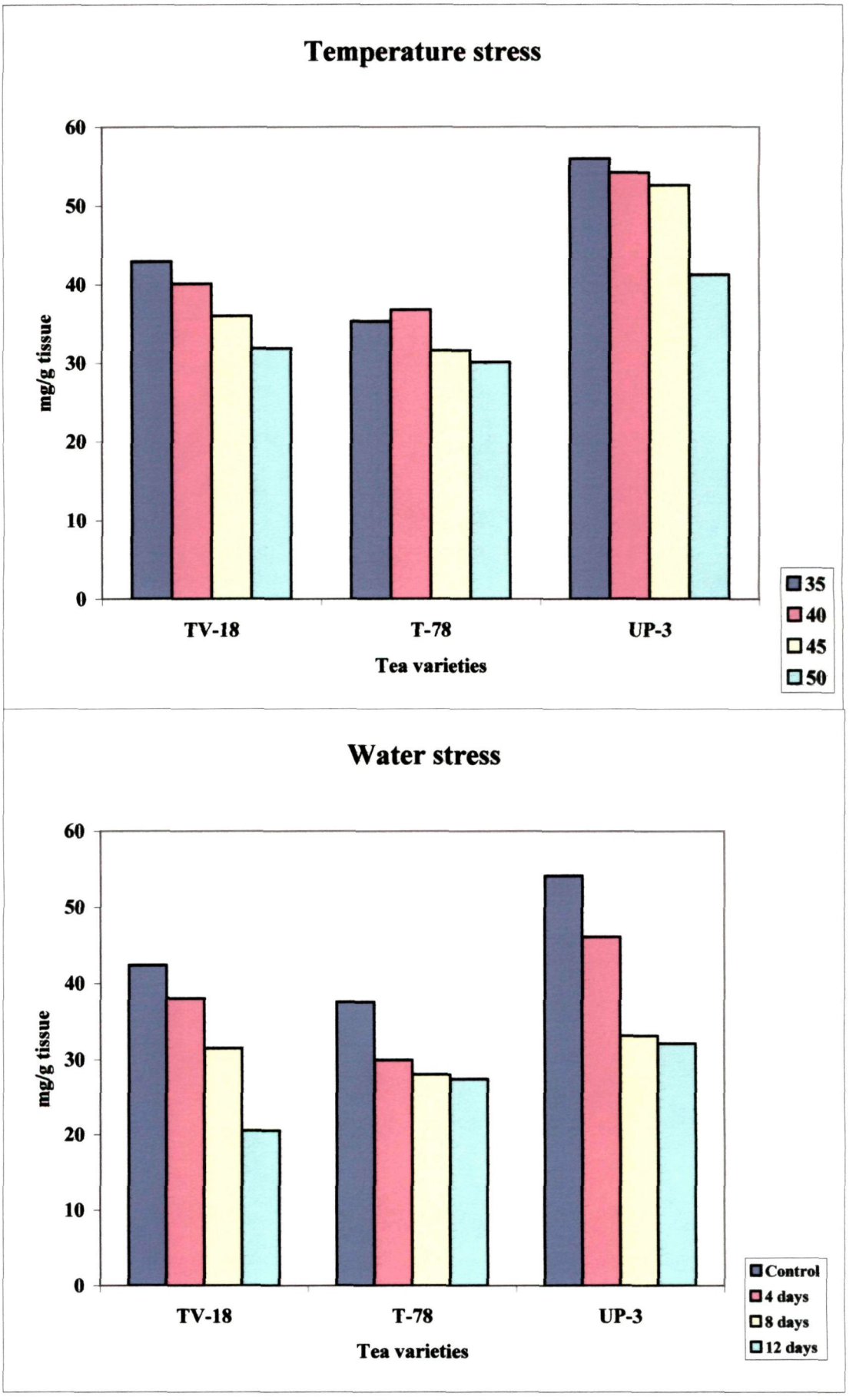


Fig.13

4.5.1.2. Anthropogenic stress

4.5.1.2.1. Fungicide

Overall decrease in protein content following spray with hexaconazole was observed which however was not very marked (Table 31). Only approximately 15% reduction of protein content was observed even after the 3rd spray.

Table 31: Effect of fungicide on protein content in tea seedlings

Tea varieties	Protein content (mg/g tissue)			
	Control	1st spray	2nd spray	3rd spray
TV-18	42.9 ± 6.21	41.7 ± 3.24	38.0 ± 0.69	36.5 ± 0.08
TV-26	44.6 ± 3.67	44.3 ± 0.09	42.8 ± 1.10	39.1 ± 1.50

C.D.(5%) for treatment mean effects = 4.61104

4.5.1.2.2. Insecticide

Result of spray with monocil was also similar to that of hexaconazole with slight reduction in protein content (Table 32).

Table 32: Effect of insecticide on protein content of young tea plants

Tea varieties	Protein content (mg/g tissue)			
	Control	1st spray	2nd spray	3rd spray
TV-18	42.9 ± 3.28	42.6 ± 5.2	40.1 ± 3.9	39.0 ± 3.08
TV-26	44.6 ± 3.27	44.3 ± 3.9	43.1 ± 2.20	40.6 ± 3.50

C.D.(5%) for treatment mean effects = 2.48756

4.5.1.3. Biotic stress

Estimation of protein content of healthy and blister infected tea leaves revealed that protein content decreased by about 33% in the infected leaves in relation to the healthy ones (Table 33), which was statistically significant. When tea leaves were artificially inoculated with spores of E. *vexans* and protein content estimated the reduction in protein content was obtained whether symptom had appeared or not.

Table 33 : Effect of blister blight infection on protein content of tea plants

Tea gardens	Protein content (mg/g tissue) Healthy leaves	Infected leaves
MarH TG	22.0 ±7.89	24.0±3.5
Dil TG	20.0±6.90	12.0±8.02
Sin TG	16.0±5.73	12.0±4.93
Cas TG	12.0±4.05	08.0±3.32
Mok TG	12.0±3.33	06.0±3.82
Mar TG	10.8±7.42	5.2±3.94
Has TG	14.5±2.1	13.2±2.2
Art.ino.	21.3±.02	15.0±.23

C.D. (5%) for treatment mean effects = 3.19805

4.5.2. SDS-PAGE analysis of proteins

Accumulation of stress induced proteins with a concomitant inhibition in synthesis of certain normal proteins are one of the most well documented response of plants to stress. In order to determine such response SDS-PAGE analysis of proteins from plants subjected to different stresses was carried out following the method described. In general, it was observed that SDS-PAGE followed by coomassie blue staining revealed a large number of protein bands varying in size from 12-115 KD. To determine the differences the protein patterns in different tea varieties was done. SDS-PAGE analysis of 12 varieties i.e. 4 UPASI varieties (UP-3, UP-9, UP-26 & BSS-2), 4 Tocklai varieties (T-17, TV-18, TV-22 & TV-26) and 4 Darjeeling varieties (T-78, B-157, HV-39 & AV-2) were carried out. Results revealed definite differences among the three groups. Most prominent of these was a protein of approximately 45 KD, which was constantly present in the Tocklai and Darjeeling varieties but was observed only in two of the four UPASI varieties. Further this band has a very high intensity in the Darjeeling varieties (Plate IX). In those varieties lacking this particular protein another protein of high molecular weight (85 KD) was lacking. High molecular weight protein of 110 KD was observed in some varieties only. The low molecular weight proteins were present in all the varieties but were prominent mainly in Darjeeling varieties and in BSS-2. Thus even among the

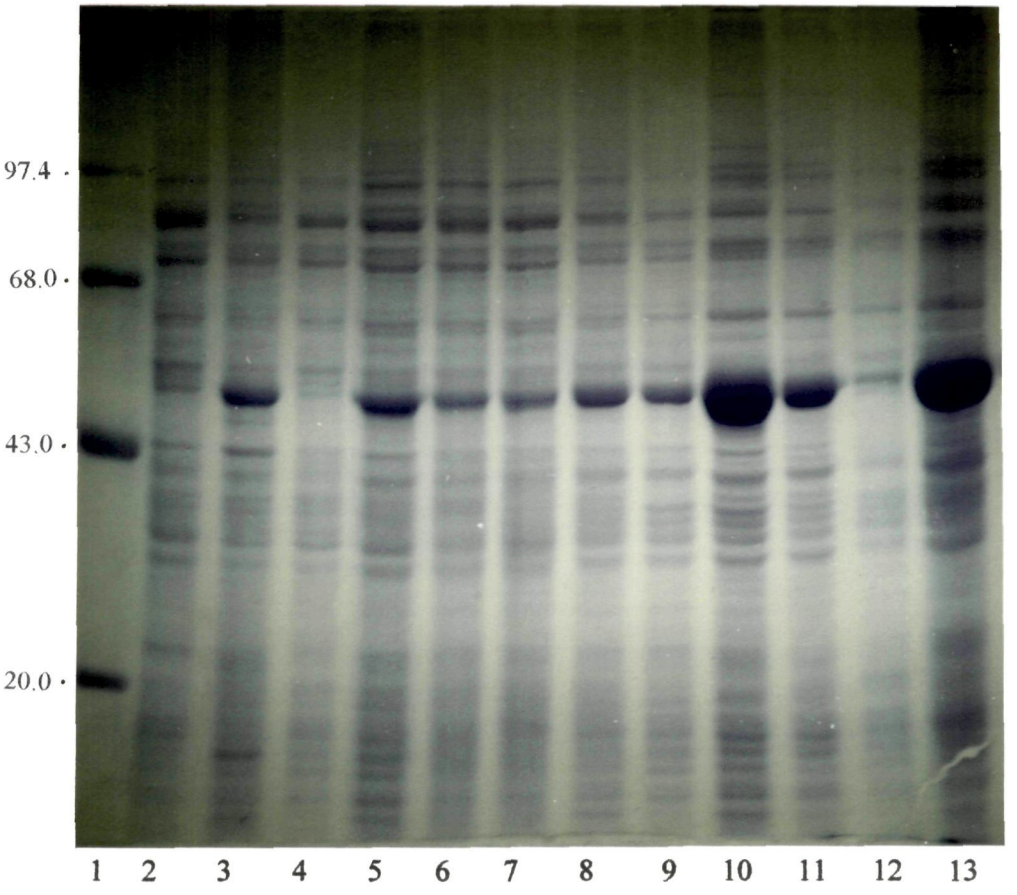


Plate IX : SDS-PAGE analysis of tea leaf proteins of healthy plants of different varieties. Lane 1- Marker, 2-UP-3, 3 -UP-9, 4-UP-26, 5-BSS-2, 6-T-17, 7-TV-18,8-TV-22, 9-TV-26, 10-T-78, 11-B-157, 12-AV-2 & 13 -HV-39

different varieties difference in protein pattern was evident. Following this changes in protein patterns following application of different stresses were determined.

4.5.2.1.Abiotic stress

4.5.2.1.1. Elevated temperature

SDS-PAGE analysis of proteins from tea leaves subjected to elevated temperatures of 40, 44, 46, 48 and 50^{0}C was carried out. Protein analysis revealed that in all the varieties no protein band could be detected on the gel from 48^{0}C onwards. In the Tocklai varieties no new protein bands could be detected in any of the elevated temperature, no protein band could be detected even at 46^{0}C. In the UPASI varieties a single new band was visible with a molecular weight approximately of 80 KD at elevated temperatures of 40^{0}Cand 44^{0}C. In the Darjeeling varieties on the other hand a new band was visible at 44 and 46^{0}C with a molecular weight of approximately 46 KD. No new high molecular weight proteins were observed in the Darjeeling varieties (Plate X).

When young tea seedlings were exposed to elevated temperatures of 40, 45 and 50^{0}C and proteins were analyzed by SDS-PAGE no new temperature induced proteins were detected in any of the varieties. In the Tocklai varieties (eg.TV-18) at 50^{0}C the number of bands decreased to only two whereas in the UPASI varieties no change was discerned even after 50^{0}C (Plate XII). In the Darjeeling varieties even at 45^{0}C the proteins bands showed significant reduction.

4.5.2.1.2. Drought

SDS-PAGE analysis of proteins from water stress and controlled plants of three varieties (UP-3, TV-18 & T-78) revealed that in all the varieties increased accumulation of proteins of molecular weights 42, 43 and 44 KD could be observed after 4 days (T-78) and 8 days (TV-18) of water stress. In TV-18 and UP-3 a new band of 74 KD appeared after 12 days of water stress. Whereas in T-78 the proteins were almost undetectable after 12 days of drought. Some of the low molecular weight protein bands ranging from14.2 to 26 KD were also more prominent following water stress (Plate XI).

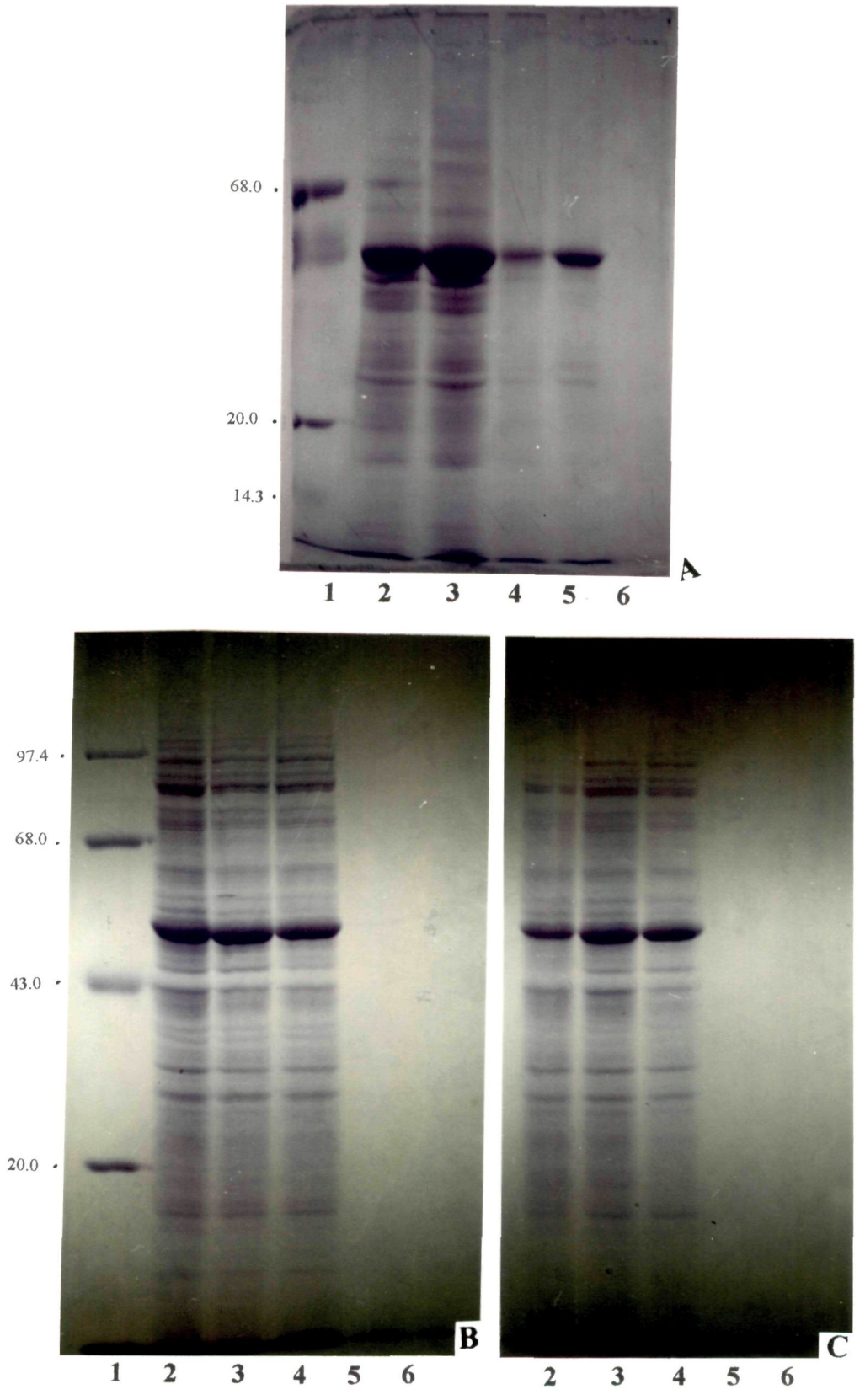


Plate X (figs.A-C) : SDS-PAGE analysis of proteins from tea leaves of different varieties subjected to temperature stress; A- T-78; B- TV-18; C-UP-3; Lane 1-Marker; 2-Control;3-40^{0}C;4-44^{0}C;5-46^{0}C;6-48^{0}C

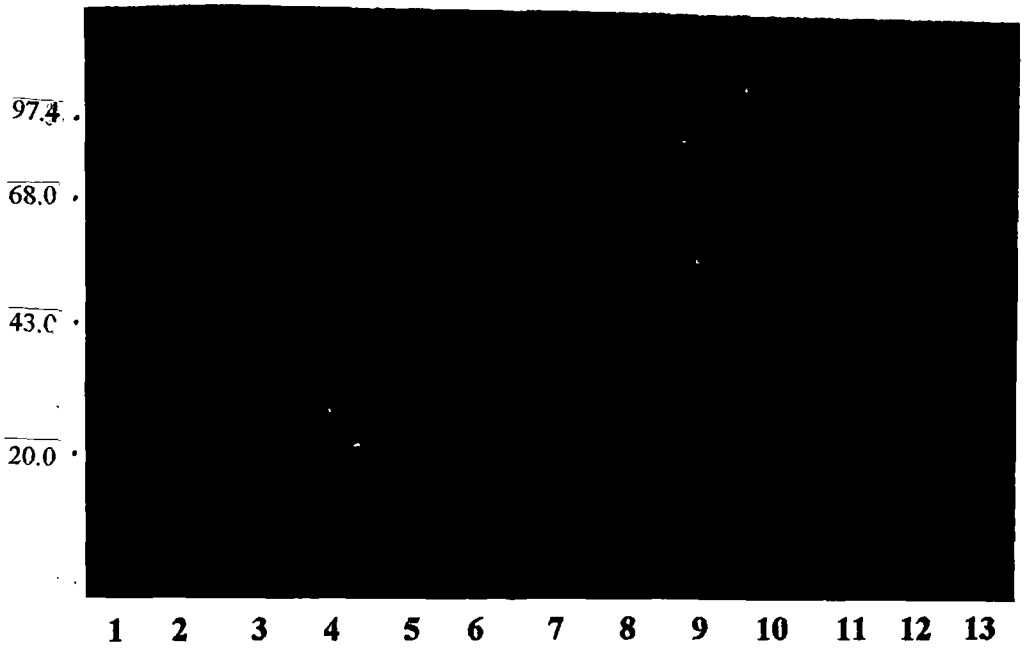


Plate XI : SDS-PAGE analysis of proteins from leaves of drought stressed tea plants of different varieties; Lane 1 – Marker; 2-5 –UP-3; 6-9-TV-18; 10-13-T-78; Lanes 2,6 &10 – Control; 3, 7& 11-4days, 4,8 & 12- 8 days and 5,9 & 13- 12 days drought

4.5.2.2. Anthropogenic

Spraying with fungicide and insecticide did not led to any change in protein pattern when analyzed by SDS-PAGE (Plate XII). No new protein could be detected even after the 3rd spray and similarly those also no loss of proteins.

4.5.2.3. Biotic stress

SDS-PAGE analysis of healthy and blister infected leaves was carried out. Different stages of blister infection were considered i.e. very young, mature and old blisters. Analysis revealed that infection led to a loss of a number of protein bands, which was very prominent in the very young and mature stages. In the old blisters reappearance of some of the protein bands was noticed with the pattern becoming closer to the healthy extracts. A new high molecular weight protein was observed in this case (Plate XII). Protein patterns of infected leaves from different tea gardens were also not different from each other.

4.6. Accumulation of proline content in tea leaves in response to different stress

Accumulation of proline in response to various stresses has been well documented. It is now well established that plants accumulate proline at an increased rate following certain stresses but its exact function is still not yet worked out in detail. In the present investigation accumulation of proline in response to the different stresses has been determined.

4.6.1. Abiotic stress

4.6.1.1. Elevated temperature

Proline content of tea leaves subjected to elevated temperatures showed an increase till 45^{0}C or 50^{0}C after which there was a decline (Table 34, Fig.14). The differences in proline content between control and temperature treatments upto 55^{0}C were statistically significant.

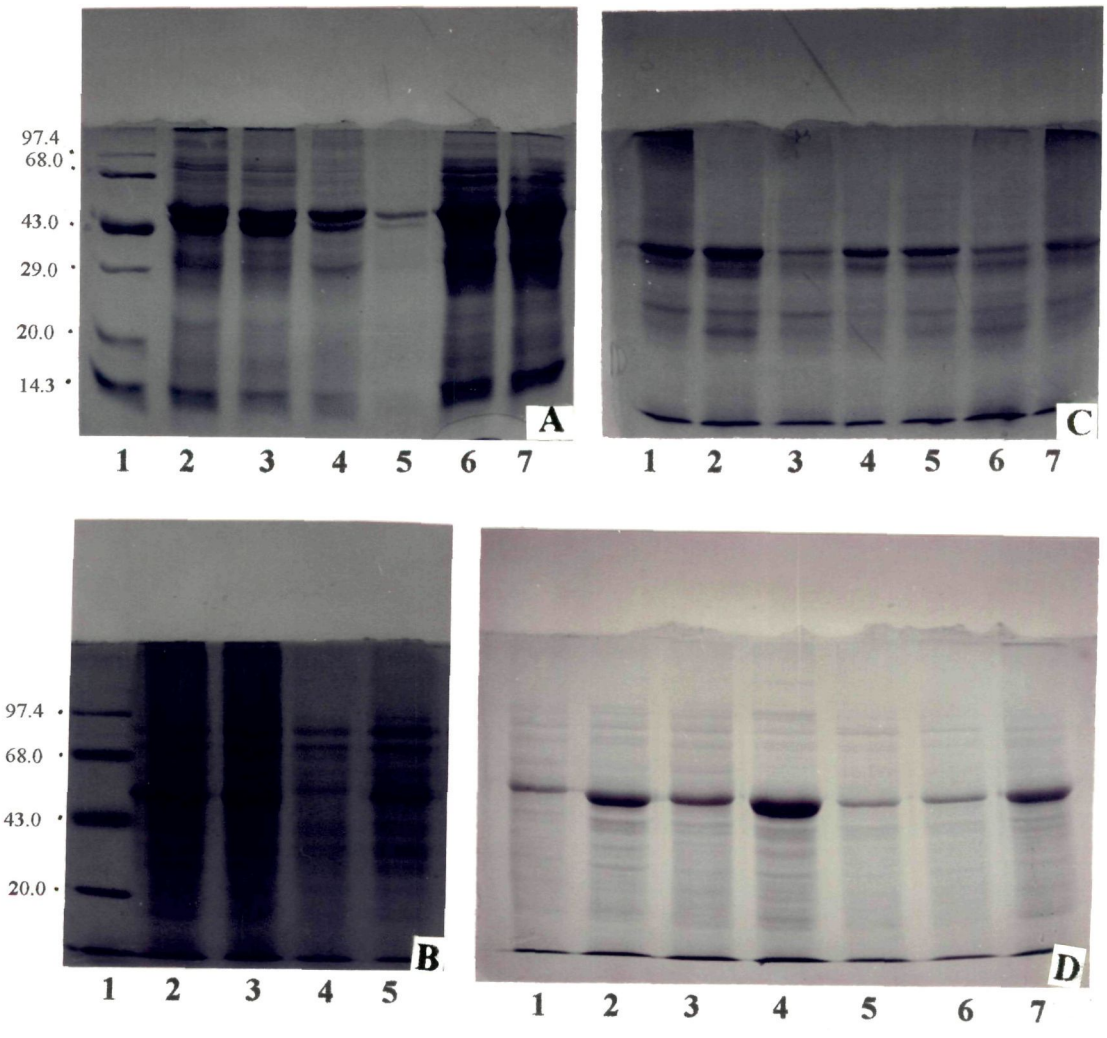


Plate XII (figs.A-D):SDS-PAGE analysis of proteins from tea plants subjected to elevated temperatures (A&B), anthropogenic stress (C) and biotic stress (D). A-TV-18:Lane 1- Marker, 2&6– Control, 3&7-40^{0}C ,4- 45^{0}C and 5-50^{0}C; B- UP-3: Lane1- Marker; 2-Control; 3-40^{0}C,4-45^{0}Cand 5-50^{0}C;C-TV-26:Lane4-Control;1-3 Fungicide & 5-7- Insecticide sprays; 1&7- 1st spray, 2&6-2nd spray and 3&5-3rd spray; D- Lanes 1-3- Blister infected leaves from Mak TG, MarH TG and Mar TG; 4-7 – Cas TG; 4-Healthy; 5-7- Young,mature and old stages of blister infection, respectively

Table 34: Effect of different temperatures on proline content in tea leaves

Varietis	Proline content (mg /g tissue) Temperatures (^{0}C)					
	Control	40	45	50	55	60
UP-2	14.2±.02	19.2±.19	23.1±.18	20.1±.14	12.3±1.2	11.1±.02
UP-3	15.2±.11	15.8±.12	20.6±.11	14.6±.22	11.1±.02	09.1±.12
UP-8	12.4±.62	25.2±.01	26.0±.14	29.6±.27	16.2±.02	12.3±.13
UP-9	11.6±.91	12.8±.18	17.6±.42	26.0±.92	20.6±.19	11.9±.38
UP-26	14.2±.10	14.9±.22	24.8±.25	31.7±.03	20.8±.92	13.0±.08
BSS-1	13.2±.12	14.5±.72	15.2±.26	16.8±.02	15.6±.42	14.0±.03
BSS-2	12.8±.16	14.7±.92	15.0±.28	17.0±1.2	13.6±.52	13.5±.24
T-17/1/54	14.4±.10	14.6±1.3	20.0±.02	13.9±.17	11.1±.27	09.9±.11
TV-9	13.2±.21	21.5±.18	22.7±.56	15.9±.81	15.6±.11	12.4±.21
TV-18	12.6±.61	15.9±.16	16.2±.17	16.0±.52	15.6±.10	13.6±.21
TV-22	13.2±1.0	14.5±.62	19.8±.16	21.8±.49	18.0±.36	23.0±74
TV-23	09.8±.03	12.0±.32	26.0±.19	11.9±.23	11.8±.23	11.6±.83
TV-25	09.6±1.0	09.9±.72	24.0±.11	12.0±.28	11.1±.03	09.0±.19
TV-26	09.0±.52	10.5±1.0	11.5±1.2	11.6±.92	11.2±.18	10.7±.02
BS/7A/76	08.3±.32	14.3±.42	14.8±.29	15.2±.28	15.3±.04	15.0±.03
T-78	09.4±.27	15.7±.82	17.6±.09	16.8±.21	16.5±.22	15.3±.02
B-157	06.3±.32	14.8±.21	16.4±.03	17.6±21	17.2±.02	15.3±.03
T-135	09.8±.21	15.4±.13	16.8±1.6	17.4±.32	16.8±.13	15.6±.02
HV-39	13.2±.22	15.2±.12	16.8±1.0	15.8±.21	14.8±.10	13.4±.01
AV-2	12.4±.12	14.6±.01	16.4±.33	16.3±.11	15.8±.01	15.3±.11

Table 34a: Analysis of variance of data presented in Table 34

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	5	833.349	166.670	16.2174	2.01131
Varieties	19	539.627	28.401	2.7635	
Error	95	976.336	10.277		
Total	119	2349.311			

Effect of elevated temperatures on proline content of tea leaves

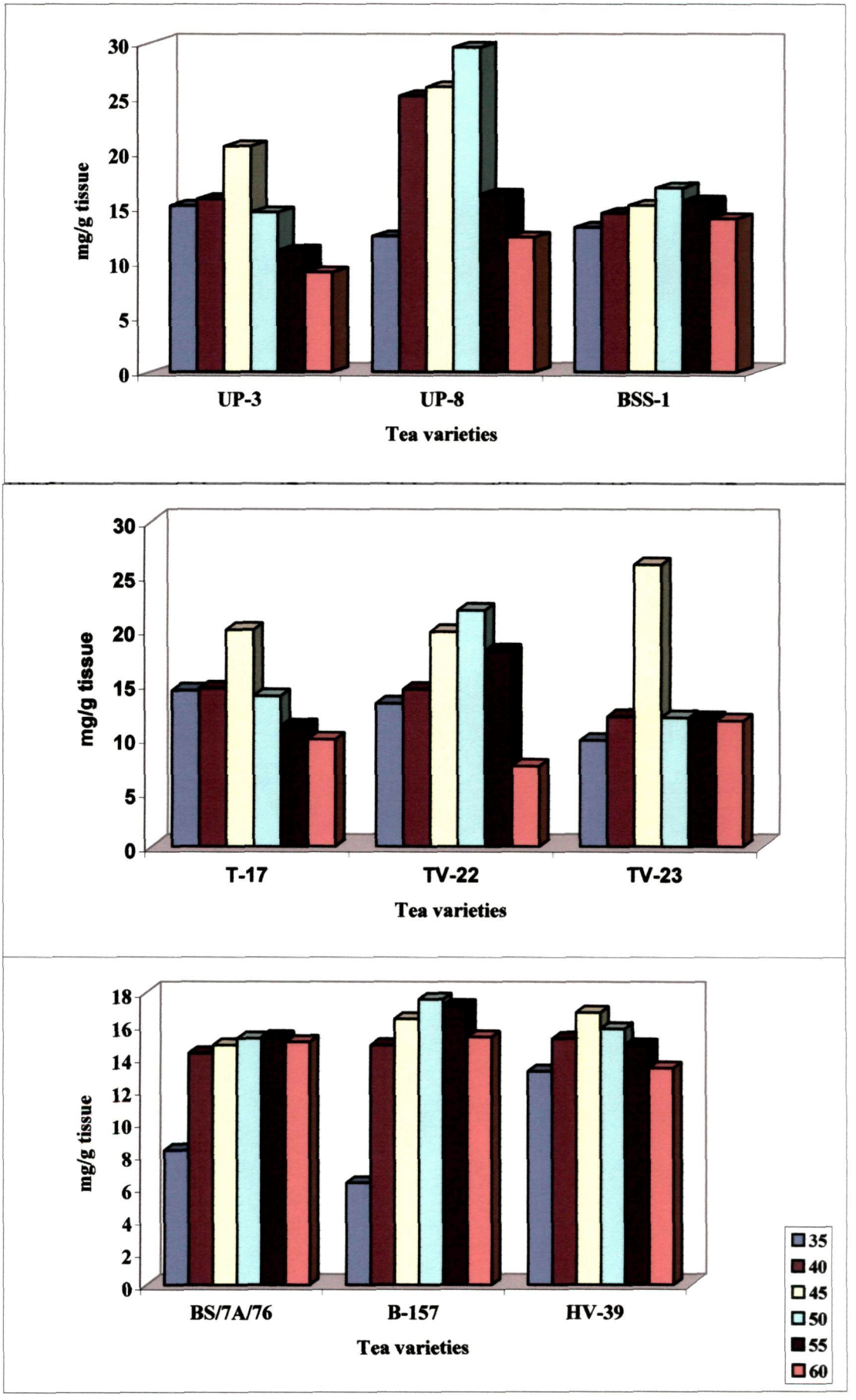


Fig.14

Wide variation in proline content was also observed among the different varieties with the proline content varying from approximately 9-15 mg/g fresh tissue. When young seedlings were exposed to elevated temperatures proline content increased till 50^0C .At high temperatures of 45 and 50^0C a highly significant increase was observed in relation to control (Table 35, Fig.15). Interestingly in this case the response of young seedlings was more significant than that of the detached leaves.

Table 35 : Effect of different temperatures on proline content of tea plants

Varieties	Proline content (mg/g tissue) Temperatures (^{0}C)			
	Control	40	45	50
T-17/1/54	4.8±1.10	8.56±1.21	10.32±1.29	11.32±2.10
TV-18	5.2 ±1.2	6.1±.04	8.8±2.34	13.5±1.1
TV-23	7.38±.02	14.2±.82	19.5±2.12	24.2±.92
TV-25	8.73±1.52	17.1±1.86	23.14±2.93	34.1±.23
TV-26	4.16±1.11	6.08±2.03	7.76±1.0	10.32±.20
T-78	6.32±.04	8.09±.56	10.6±.34	14.56±.01
HV-39	7.61±.48	12.8±2.01	19.09±.82	24.4±.22
UPASI-3	1.34±.01	1.78±.72	4.40±.68	7.33±.21
UPASI-9	1.55±.03	1.95±.92	4.48±.38	9.97±.36
BSS-2	2.02±.24	2.53±.23	7.13±.23	17.36±.32

Table 35a: Analysis of variance of data presented in Table 35

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	3	771.980	257.327	29.9669	3.10981
Varieties	9	1124.943	124.994	14.5561	
Error	27	231.850	8.587		
Total	39	2128.772			

4.6.1.2. Drought

Water stress induced increased accumulation of proline. After 4 days the increase in proline content was not very significant but after 8 and 12 days proline content increased drastically in all the varieties (Table 36). After 8 days of water stress proline content had increased by approximately 10 fold whereas after 12 days the increase was approximately 50 fold or more. The increase was maximum in case of

Effect of elevated temperatures on proline content of tea seedlings

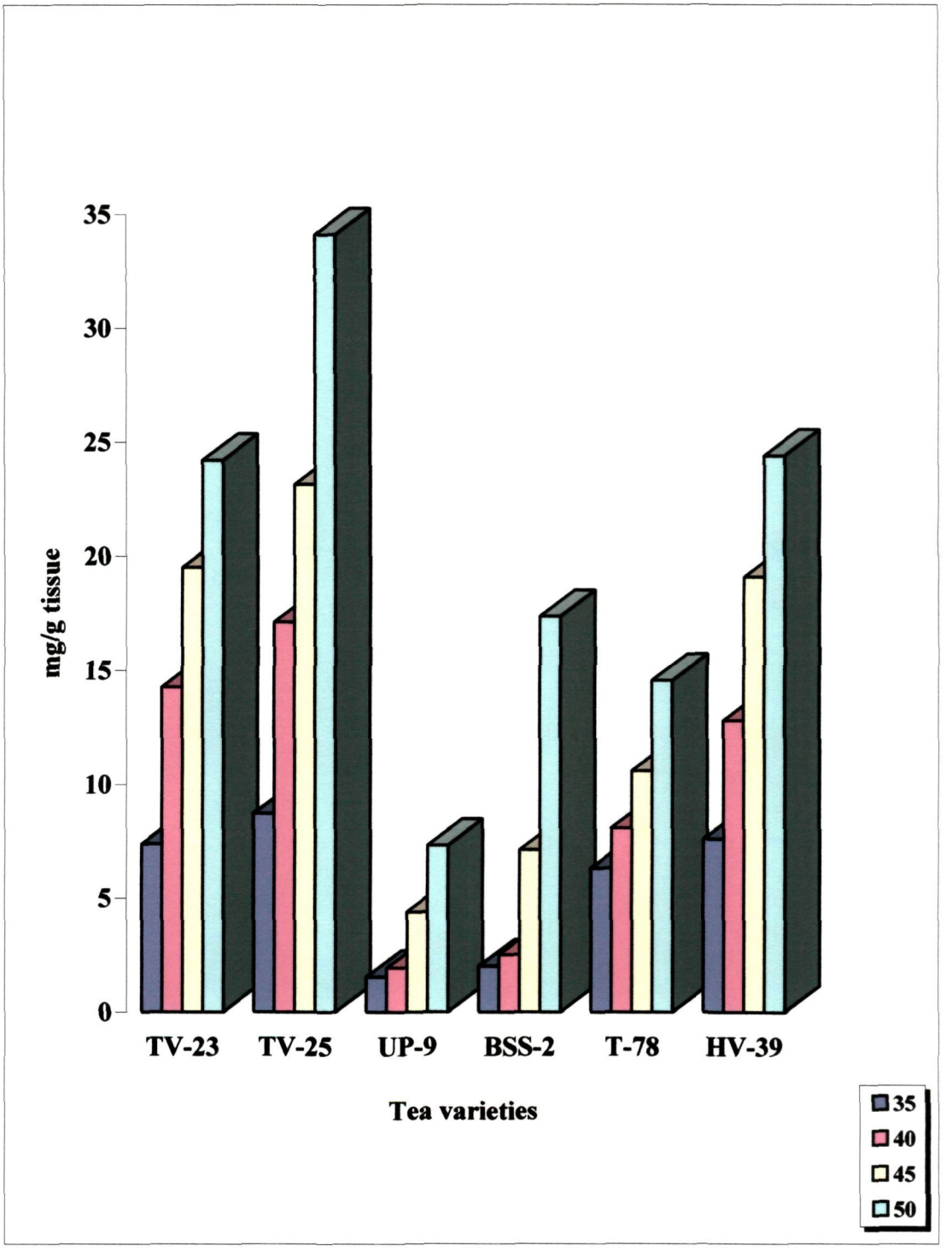


Fig.15

Darjeeling varieties, though all varieties showed a significant increase.

Table 36: Effect of water stress on proline content in tea plants

Tea variety	Proline content (mg/g tissue) Days of water stress			
	Control	4	8	12
TV-18	4.38±.03	6.13±.32	12.6±.20	24.10±.24
TV-26	4.14±.58	4.36±.28	16.28±.42	55.67±.23
UPASI-3	1.40±.34	2.54±.08	22.36±.18	43.84±.78
UPASI-26	2.10±.32	2.46±.26	13.25±.08	48.25±.10
T-78	6.52±.21	7.55±.34	12.17±.28	84.40±.16
HV-39	7.13±.12	9.19±.02	11.45±.28	145.9±.22

Table 36a:Analysis of variance of data presented in Table 36

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	3	16011.86	5337.28	11.5083	30.95945
Varieties	5	2576.321	515.264	1.1110	
Error	15	6956.651	463.777		
Total	23	25544.84			

4.6.2. Anthropogenic stress

4.6.2.1. Fungicide

An increase in proline content following spray with haxaconazole was observed, which was statistically significant at 2nd and 3rd spray. After the 3rd spray in TV-18 increase was about 3 fold whereas in TV-26 it was about 2 fold (Table 37. Fig.16).

Table 37: Effect of fungicide on proline content of young tea plants

Tea varieties	Proline content (mg/g tissue)			
	Control	1st spray	2nd spray	3rd spray
TV-18	4.90 ± 0.20	5.48± 0.20	7.19 ± 0.9	9.06 ± 0.8
TV-26	4.26 ± 0.17	4.6 ± 0.90	7.56 ± 0.2	10.1 ± 0.5

C.D. (5%) for treatment mean effects = 2.64084

4.6.2.2. Insecticide

Spraying with the insecticide also induced a greater accumulation of proline which was more significant than that of hexaconazole. In TV-18 a highly significant increase of about 10 fold was observed following the 3rd spray (Table 38, Fig. 16).

Table 38: Effect of insecticide on proline content of young tea plants

Tea varieties	Proline content (mg/g tissue)			
	Control	1st spray	2nd spray	3rd spray
TV-18	4.9 ±0.8	5.8 ± 0.2	8.53 ± .39	16.37 ± .18
TV-26	4.24 ±0.7	4.61 ± 0.9	6.39 ± .21	8.01 ± .14

C.D. (5%) for treatment mean effects = 10.53565

4.6.3. Biotic stress

Blister infection led to a significant increase in proline content. In general the increase following infection was 44% (Table 39, Fig.16). Artificially inoculated leaves also exhibited increased accumulation of proline in comparison to the healthy leaves.

Table 39: Effect of blister blight infection on proline content in tae plants

Tea gardens	Proline content (mg/g tissue)	
	Healthy leaves	Infected leaves
MarH TG	6.48±2.21	8.24±2.26
Dil TG	5.12±2.09	7.24±1.24
Sin TG	8.00±1.98	9.48±1.11
Cas TG	6.28±2.54	8.72±2.0
Mok TG	8.40±1.62	12.16±.54
Mar TG	6.48±.98	12.00±2.26
Has TG	4.1±1.11	13.0±3.02
Art.ino.	2.33±.09	3.63±.40

Critical difference for treatment mean effects = 2.58923

4.7. Effect of different stresses on phenolics in tea leaves

Polyphenols are the major components of tea leaves and hence it is expected that any stress to the plant would cause in the changes in the phenolics. Phenols are also known to play definite roles in a plant defense. Considering this in the

Effect of anthropogenic and biotic stress on proline content of tea seedlings

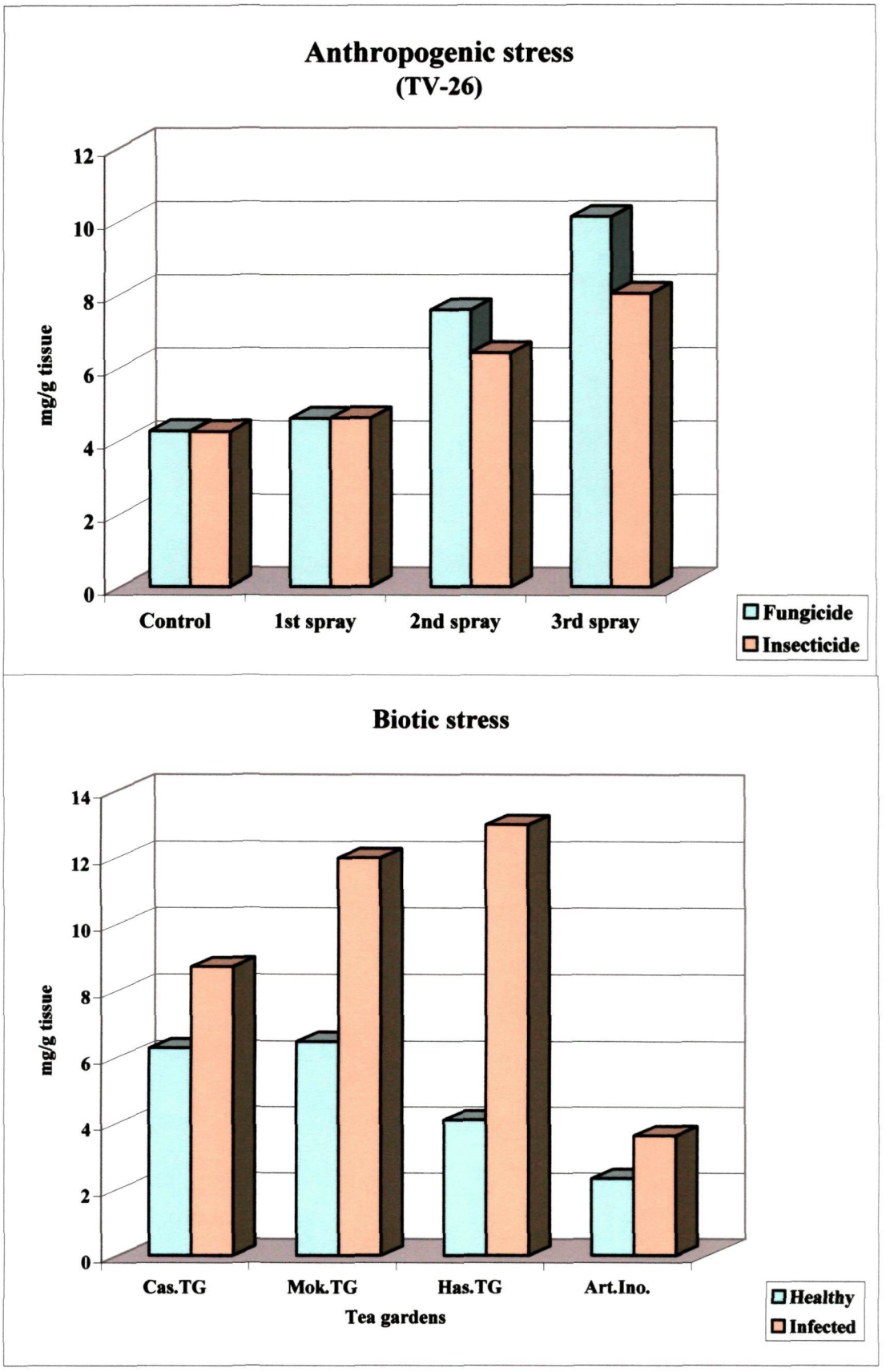


Fig.16

present study the effect of the different stresses on phenol contents were determined. Further experiments were also carried out to detect the antifungal phenolics in the tea leaves before and after being subjected to stress.

4.7.1. Phenol content

Total phenols were extracted and estimated following the method described under materials and methods. Besides the content of O-dihydroxy phenols, which is an important constituent of the total phenols in plants were also examined.

4.7.1.1. Abiotic stress

4.7.1.1.1. Elevated temperatures

For studies on phenolics in detached leaves, temperature treatments were applied by keeping the leaves in water tight containers immersed in water bath at desired temperature. This was done to avoid loss of diffusable phonolics from the leaves. Estimation of total phenol revealed that significant differences could be observed among the different varieties ranging from approximately 12-45 mg/g fresh wt. of tissue. Elevated temperature led to an increase in phenol contents in only eight out of twenty varieties in detached leaf treatment (Table 40). In general, significant difference in phenol content of temperature stressed leaves in relation to control was obtained from 50^0C onwards.When all the Darjeeling varieties and most of the Tocklai varieties elevated temperature led to a decrease in phenol content. In varieties with significantly high inherent phenol content was no increase of total phenol content after temperature elevation (Fig. 17). In case of O-dihydroxy phenol content the trend was similar to that of total phenol content with the content decreasing with elevated temperature in most of the va rieties (Table 41, Fig. 17).

Among the ten varieties of young tea seedlings exposed to different elevated temperatures it was observed that changes in the phenol content was dependent on the inherent phenols. In varieties with high inherent phenol (UPASI-3- 34 mg, BSS-2- 36mg) elevated temperatures did not induce any increase. On the other hand varieties with low inherent phenols (T-78- 9mg, T-17- 10.5mg) phenol contents increased

Table 40:Effect of different temperatures on total phenol content of tea leaves

Varieties	Phenol content (mg /g tissue) Temperatures (^{0}C)					
	Control	40	45	50	55	60
UP-2	11.7±.02	23.0± .02	23.7 ±21	23.7. ±21	20.2.±.16	18.8 ±04
UP-3	16.3.±.02	23.4.±.12	30.0.±.04	25.5.±.08	23.8.±.14	22.5.±.20
Up-8	15.0.±.09	23.7.±.18	31.0.±.19	28.6.±.13	18.7.±.14	16.5.±.12
UP-9	35.0.±.17	38.3.±.02	43.3.±.09	31.4.±.03	25.0.±.31	23.8.±.15
UP-26	35.0.±.09	30.1.±.25	28.3.±.16	26.2.±.21	25.0.±.15	22.2.±.11
BSS-1	28.0.±.05	31.3.±.04	43.3.±.18	31.4.±.03	25.2.±.16	23.4.±.15
BSS-2	30.2.±.12	35.0.±.11	38.3.±.21	30.0.±.07	27.5.±.04	23.0.±.04
T-17/1/54	23.0.±.09	22.5.±.19	20.0.±.01	15.6.±.11	12.0.±.03	12.0.±.06
TV-9	28.3.±.24	23.8±.04	23.0.±.08	22.2.±.12	18.8.±.09	16.7.±.08
TV-18	23.0.±.23	24.5.±.32	27.5.±.14	26.3.±.26	18.8.±.06	16.7.±.08
TV-22	26.0.±.08	28.9.±.05	31.5.±.12	23.2.±.14	20.0.±.12	12.5.±.02
TV-23	43.2.±.09	38.7.±.13	32.0.±.08	25.5.±.07	23.0.±.17	19.0.±.02
TV-25	43.0.±.17	38.3.±.08	31.4.±.03	28.8.±.09	20.0.±.08	19.0.±.02
TV-26	45.0.±.09	36.5.±.13	26.0.±.23	25.9.±.22	18.0.±.09	10.3.±.15
BS/7A/76	16.3.±.09	11.8.±.11	11.5.±.08	11.3.±.07	10.0.±.01	08.1.±.01
T-78	23.5.±.15	21.3.±.09	18.0.±05	15.5.±.23	15.0.±.09	14.0.±.13
B-157	37.5.±.09	31.5.±.18	26.3.±.24	23.8.±.17	22.5.±.08	22.0.±.06
T-135	35.0.±.09	26.8.±.07	26.5.±.15	23.2.±.03	22.5.±.05	21.2.±.02
HV-39	42.5.±.12	28.3.±.08	23.8.±.05	19.5.±.01	18.8.±.14	15.0.±.02
AV-2	35.0±.14	31.5±.12	26.3±.14	23.8±.06	22.5±.13	19.0±.02

Table 40a: Analysis of varience of data presented in Table 40

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	5	2280.233	456.047	20.0268	2.99392
Varieties	19	3045.846	160.308	7.0398	
Error	95	2163.332	22.772		
Total	119	7489.399			

Table 41: Effect of different temperatures on O- dihydroxy phenol content of tea leaves.

Varieties	O- dihydroxy phenol content (mg/g tissue) Temperatures (^{0}C)					
	Control	40	45	50	55	60
UP-2	03.2±.07	0.05±.04	06.7±.13	06.7±.11	04.6±.01	04.1±.20
UP-3	03.5±.25	03.9±.21	04.2±.05	04.1±.14	03.9±.01	03.5±.08
UP-8	03.4±.06	07.1±.12	08.7±.16	08.1±.21	05.3±.08	05.0±.03
UP-9	06.3±.15	06.9±.21	08.3±.16	07.1±.26	06.7±.09	03.2±.03
UP-26	07.9±.17	07.4±.20	06.7±.08	05.9±.14	05.2±.07	05.0±.03
BSS-1	06.7±.21	08.0±.14	10.3±.17	08.2±.11	07.2±.12	07.1±.03
BSS-2	07.9±.32	09.6±.09	10.2±.07	07.0±.18	06.4±.12	06.0±.05
T-17/1/54	07.3±.10	06.9±.09	06.4±.23	04.2±.05	03.7±.02	03.2±.01
TV-9	06.7±.19	09.5±.13	09.6±.15	07.5±.11	05.0±.09	04.2±.03
TV-18	06.5±.02	06.8±.12	07.3±.19	07.1±.09	05.1±.17	03.7±.16
TV-22	06.3±.12	07.5±.06	10.3±.13	06.2±.14	06.0±.17	03.9±.12
TV-23	13.0±.17	11.6±.17	08.3±.09	08.4±.23	07.1±.16	05.7±.13
TV-25	14.5±.11	12.5±.09	10.5±.21	08.2±.24	06.1±.19	05.3±.12
TV-26	08.2±09	08.1±.04	07.7±.12	04.5±.05	04.3±.12	03.7±.11
BS/7A/76	05.7±.07	05.5±.12	05.0±.15	05.2±.13	04.9±.16	03.1±.11
T-78	05.9±.23	04.1±.10	05.0±.10	04.3±.07	04.1±.04	04.0±.02
B-157	10.3±.11	09.3±.12	08.3±.19	06.7±.09	06.8±.12	05.7±.12
T-135	11.5±.07	08.8±.18	08.5±.13	06.7±.13	05.3±.10	05.2±.11
HV-39	12.5±.17	09.2±.12	08.8±.07	08.3±.10	06.0±.15	04.2±.23
AV-2	11.0±.09	08.7±.03	07.3±.21	07.3±.13	07.0±.01	06.3±.02

Table 41a: Analysis of varience of data presented in Table 41

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	5	196.713	39.343	20.0058	0.87982
Varieties	19	267.081	14.057	7.1479	
Error	95	186.824	1.967		
Total	119	650.618			

Effect of elevated temperatures on phenol content of tea leaves

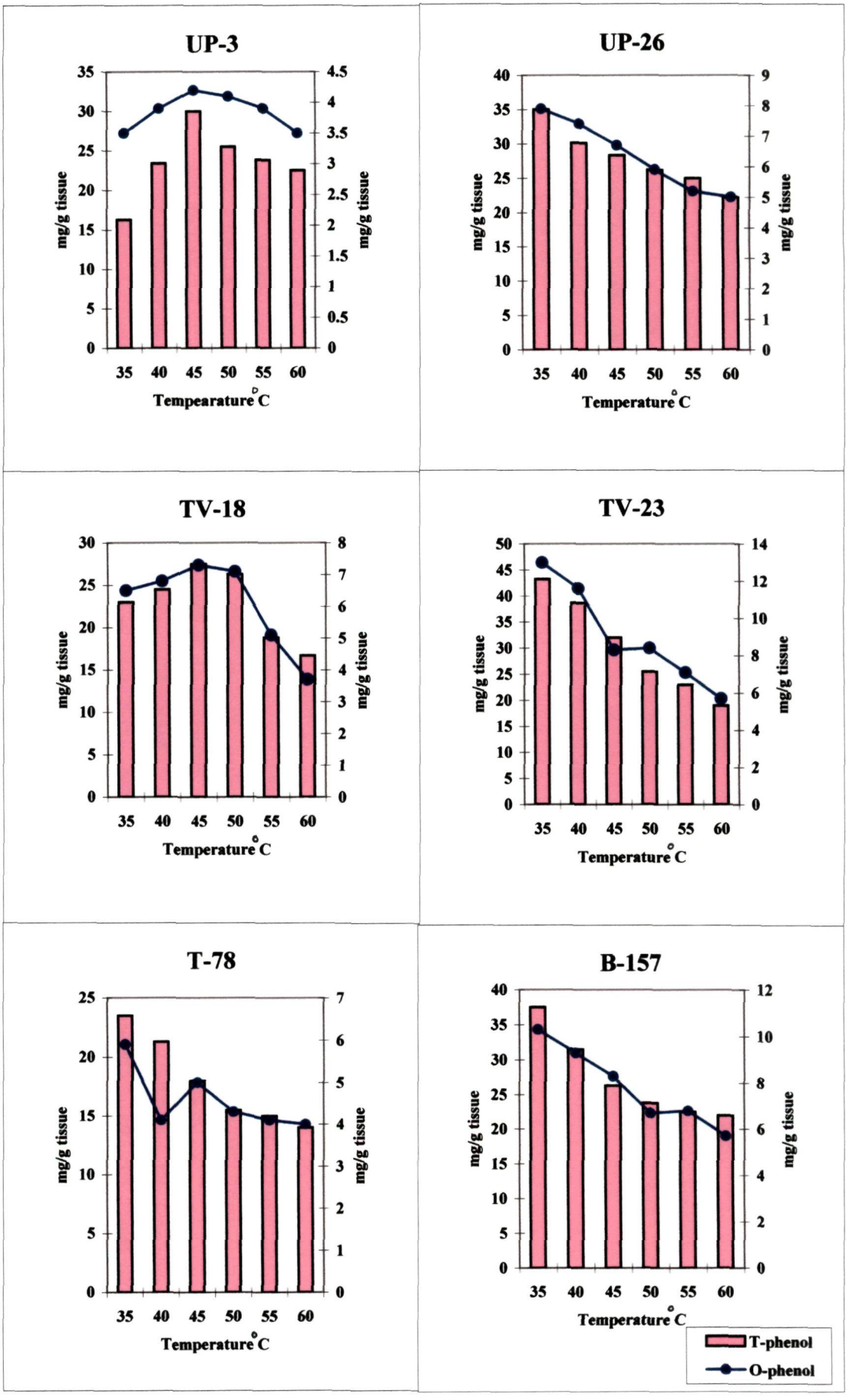


Fig.17

Table 42:Effect of different temperatures on phenol content young tea plants

Varieties	Phenol content (mg /g tissue) Temperature (^{0} C)			
	Control	40	45	50
T-17/1/54	10.5±1.18	20.5±4.3	24.5±.32	29.3±.22
TV-18	20.0±1.92	23.8±2.3	38.4±.56	32.6±.2.1
TV-23	31.8±.98	16.2±4.6	16.7±1.2	16.5±.2.8
TV-25	23.8±.47	25.4±2.2	20.5±2.8	14.9±.3.4
TV-26	10.8±.22	12.5±3.0	21.0±1.2	35.5±1.3
T-78	9.00±.21	27.8±1.43	20.5±.82	21.5±.32
HV-39	14.6±.09	32.5±.83	32.3±.86	20.8±.96
UP-3	33.9±1.2	19.4±.56	16.4±.21	12.5±.53
UP-9	17.0±.21	24.2±.22	24.7±.31	20.6±.26
BSS-2	36.0±1.2	31.9±.08	18.21±.22	17.2±.12

Table 42a: Analysis of variance of data presented in Table 42

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	3	143.727	47.909	0.7555	8.45084
Varieties	9	506.963	56.329	0.8883	
Error	27	1712.136	63.412		
Total	39	2362.826			

significantly with increase in temperature. Other varieties showed maximum phenol accumulation at 40^{0}C or 45^{0}C (Table 42, Fig.18).O-dihydroxy phenols also showed similar trends (Table 43, Fig. 18).

Table 43: Effect of different temperature on O-dihydroxy phenol content of young tea plants

Varieties	O-dihydroxy Phenol content (mg/g tissue) Temperatures (^{0} C)			
	Control	40	45	50
T-17/1/54	3.7±.02	10.0±.34	9.0±.02	10.56±.12
TV-18	5.2±.21	5.5±.82	10.7±.22	7.6±.14
TV-23	1.6±.64	1.8±1.8	2.8±.31	3.1±.04
TV-25	2.53±.17	3.52±.52	3.43±.46	3.2±.08
TV-26	2.25±.1.6	2.5±1.28	9.5±.26	12.5±.24
T-78	2.0±.02	8.0±.24	9.5±.18	8.45±.28
HV-39	2.51±.14	3.09±.82	5.0±.18	4.56±.12
UP-3	9.95±.21	4.61±1.32	3.26±.12	3.02±.18
UP-9	3.39±.45	5.23±.25	7.01±.82	2.16±.20
BSS-2	7.20±.12	5.97±.86	4.57±.16	2.18±.22

Table 43a: Analysis of variance of data presented in Table 43

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	3	32.773	10.924	1.5138	2.85080
Varieties	9	136.071	15.119	2.0951	
Error	27	194.838	7.216		
Total	39	363.682			

4.7.1.1.2. Drought

Both total and O-dihydroxy phenols are showed increased accumulation following 4 and 8 days of water stress after which there was a decline in prolonged water stress. Maximum increase was observed after 8 days of water stress, which was statistically significant (Table 44, Fig.19).

Table 43: Effect of different temperature on O-dihydroxy phenol content of young tea plants

Varieties	O-dihydroxy Phenol content (mg/g tissue) Temperatures (^{0} C)			
	Control	40	45	50
T-17/1/54	3.7±.02	10.0±.34	9.0±.02	10.56±.12
TV-18	5.2±.21	5.5±.82	10.7±.22	7.6±.14
TV-23	1.6±.64	1.8±1.8	2.8±.31	3.1±.04
TV-25	2.53±.17	3.52±.52	3.43±.46	3.2±.08
TV-26	2.25±.1.6	2.5±1.28	9.5±.26	12.5±.24
T-78	2.0±.02	8.0±.24	9.5±.18	8.45±.28
HV-39	2.51±.14	3.09±.82	5.0±.18	4.56±.12
UP-3	9.95±.21	4.61±1.32	3.26±.12	3.02±.18
UP-9	3.39±.45	5.23±.25	7.01±.82	2.16±.20
BSS-2	7.20±.12	5.97±.86	4.57±.16	2.18±.22

Table 43a: Analysis of variance of data presented in Table 43

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	3	32.773	10.924	1.5138	2.85080
Varieties	9	136.071	15.119	2.0951	
Error	27	194.838	7.216		
Total	39	363.682			

4.7.1.1.2. Drought

Both total and O-dihydroxy phenols are showed increased accumulation following 4 and 8 days of water stress after which there was a decline in prolonged water stress. Maximum increase was observed after 8 days of water stress, which was statistically significant (Table 44, Fig.19).

Effect of elevated temperatures on phenol content of tea seedlings

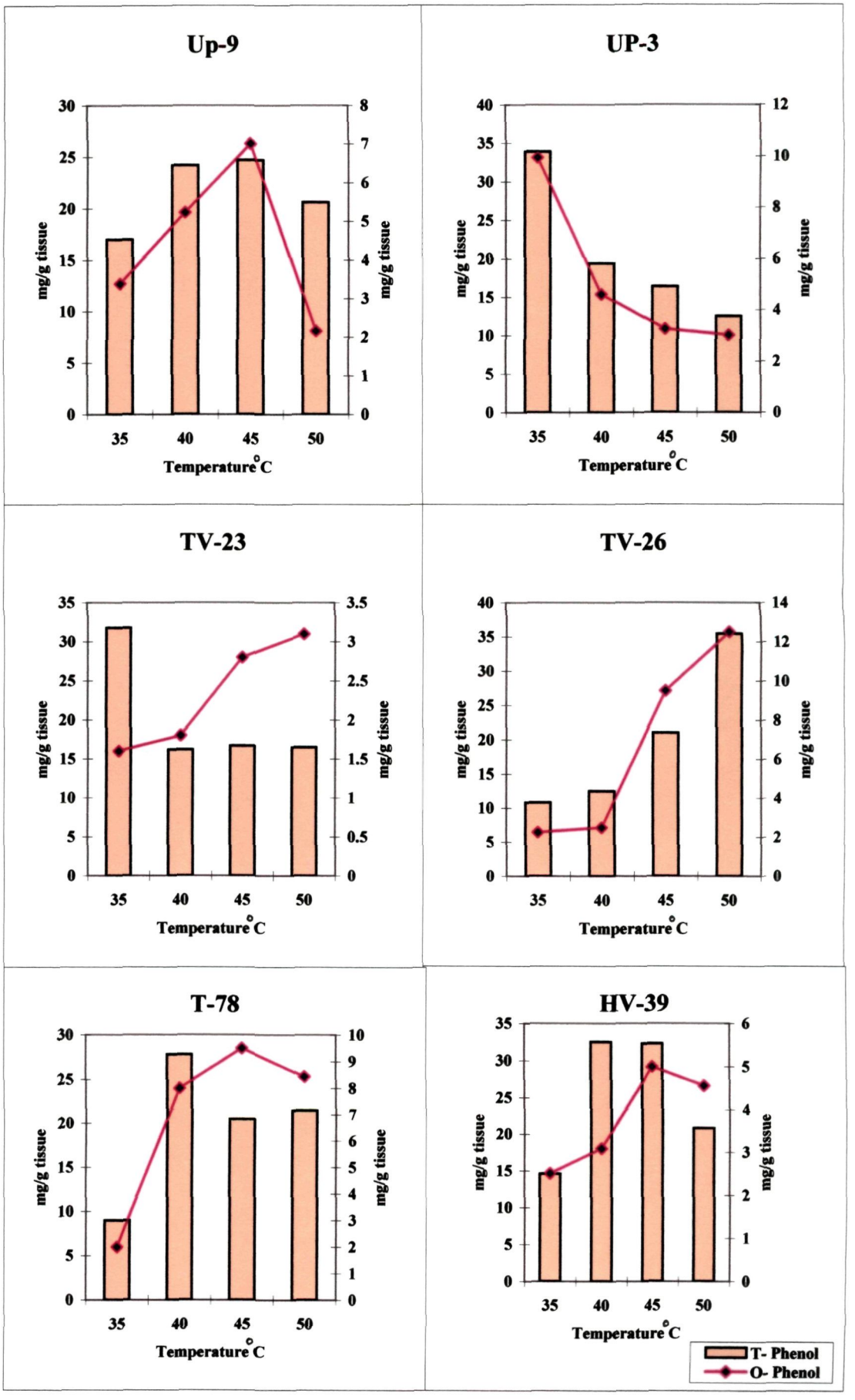


Fig.18

Table 44: Effect of water stress on phenol content of young tea plants

Tea variety	Phenol content (mg/g tissue) Days of water stress			
	Control	4	8	12
TV-18	19.2±3.02	22.82±2.18	24.93±3.32	13.11±1.20
TV-26	14.6±2.83	27.66±3.28	32.04±2.36	15.16±2.68
UP-3	32.9±3.14	36.08±3.22	39.10±4.38	32.8±2.44
UP-26	18.96±2.18	24.60±4.12	26.97±2.46	24.60±2.10
T-78	10.20±2.11	28.00±2.22	40.00±5.01	12.68±2.2
HV-39	16.21±4.10	22.74±1.28	28.49±3.02	11.02±1.01

Table 44a: Analysis of varience of data presented in Table 44.

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	3	798.870	266.290	11.9145	6.79639
Varieties	5	660.584	132.117	5.9112	
Error	15	335.251	22.350		
Total	23	1794.705			

Table 45: Effect of water stress on O-dihydroxy phenol contents of young tea Plants

Tea variety	Ortho dihydroxy phenol (mg/g tissue) Days of water stress			
	Control	4	8	12
TV-18	3.12±1.12	3.65±.82	4.53±.62	2.68±.24
TV-26	2.61±.21	3.84v1.22	7.2±1.2	3.12±.3828
UPASI-3	3.86±2.18	4.91±2.03	5.26±2.03	3.26±.92
UPASI-26	2.82±1.92	4.33±2.14	5.90±2.11	2.98±1.01
T-78	2.12±.98	3.46±1.14	10.02±2.16	3.63±.02
HV-39	2.16±2.02	2.84±.42	5.85±.04	1.75±.91

Table 45a: Analysis of variance of data presented in Table 45

Source	D.F.	S.S.	M.S.	F	C.D.(5%)
Treatment	3	52.588	17.529	14.0043	1.60839
Varieties	5	7.082	1.416	1.1316	
Error	15	18.776	1.252		
Total	23	78.446			

4.7.1.2. Anthropogenic stress

4.7.1.2.1. Fungicide

Both total and O-dihydroxy phenol contents increased due to fungicide spray. In both varieties maximum accumulation was observed following 2nd spray, which was statistically significant. The phenolic content even after 3rd spray was higher than that of control (Table 46 & 47, Fig.19).

Table 46: Effect of fungicide on total phenol content in tea plants

Tea varieties	Phenol content (mg/g tissue)			
	Control	1st spray	2nd spray	3rd spray
TV-18	24.27 ± 1.20	29.55± 1.20	33.42 ± 1.9	30.16± 2.8
TV-26	13.15 ± 3.17	42.75± 1.90	48.63 ± 2.2	39.18 ± 2.5

C.D.(5%)for treatment mean effects = 35.65578

Table 47: Effect of fungicide on O-dihydroxy phenol content in tea plants

Tea varieties	**Phenol content (mg/g tissue)**			
	Control	**1st spray**	**2nd spray**	**3rd spray**
TV-18	6.8± 2.28	6.8 ± 0.48	9.6 ± 0.86	8.8± 0.12
TV-26	8.23 ± 1.7	9.01 ± 0.934	11.24 ± 2.2	9.6 ± 0.3

C.D. (5%) for treatment mean effects = 1.799

4.7.1.2.2. Insecticide

Spraying with monocil also led to an increase in O-dihydroxy and total phenol content in both of the varieties, the maximum being after the 2nd spray (Table 48 & 49).

Effect of water stress and anthropogenic stress on phenol content of tea seedlings

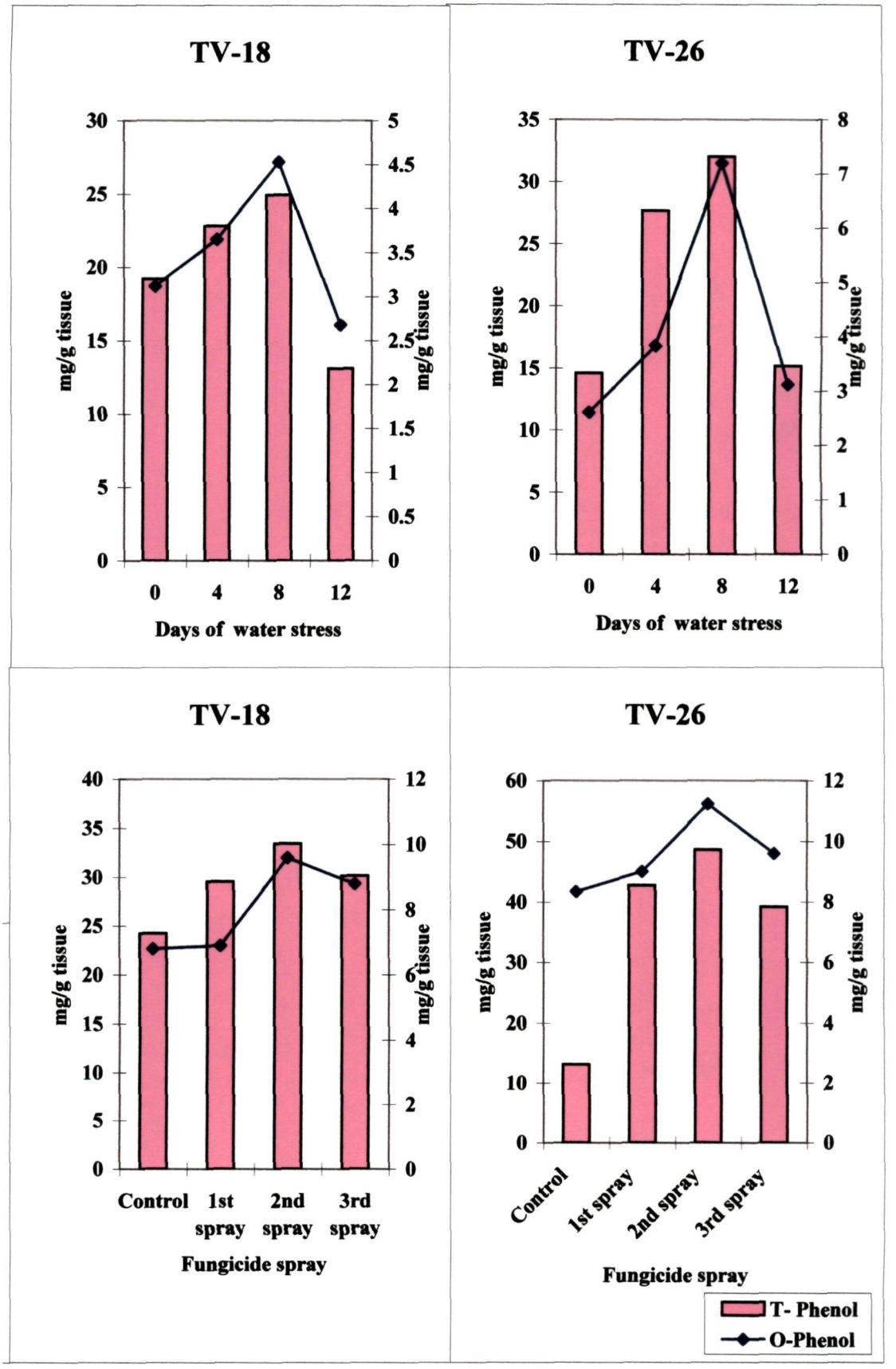


Fig.19

Table 48: Effect of insecticide on total phenol content in tea plants

Tea varieties	Total Phenol content (mg/g tissue)			
	Control	1st spray	2nd spray	3rd spray
TV-18	24.27 ± 0.20	40.45± 0.20	48.07 ± 0.9	21.18 ± 0.8
TV-26	13.15 ± 0.17	44.82 ± 0.90	55.09 ± 0.2	28.06 ± 0.5

C.D. (5%) for treatment mean effects = 25.66256

Table 49 :Effect of insecticide on O-dihydroxy phenol content of tea plants

Tea varieties	O-dihydroxy Phenol content (mg/g tissue)			
	Control	1st spray	2nd spray	3rd spray
TV-18	6.8 ± 2.28	7.1± 2.40	10.3± 6.9	24.1± 3.8
TV-26	8.23 ± 1.7	9.68 ± 1.90	12.6 ± 0.78	16.2± 0.9

C.D. (5%) for treatment mean effects = 14.839

4.7.1.3. Biotic stress

Blister blight infection also led to a significant increase in both total and O-dihydroxy phenol content of tea leaves (Table 50).

Table 50: Effect of blister blight infection on phenol content of tea plants

Tea gardens	Healthy leaves		Infected leaves	
	Total phenol (mg/g tissue)	O dihy. Phenol (mg/g tissue)	Total phenol (mg /g tissue)	O dihy. Phenol (mg / g tissue)
MarH TG	36±1.18	2.50±.98	45±.4.1	4.20±2.01
Dil TG	34.2 ±1.82	2.5±.34	54±4.92	4.2 ± 2.2
Sin TG	34.2±1.36	3.75±.36	46.2±3.68	4.0±.04
Cas TG	52.2±2.08	4.2±.78	58.8±2.26	4.4±.47
Mok TG	54.0±3.28	4.0±.22	59±2.94	4.8±.34
Mar TG	28.9±3.88	2.25±1.0	46.2±2.08	4.2±.22
Has TG	36.3±2.21	4.83±1.8	41.2±1.86	7.82±.28

C.D. (5%) for treatment mean effects for total phenol = 5.11484

C.D. (5%) for treatment mean effects for O-dihydroxy phenol = 0.92529.

4.7.2. Antifungal phenolics

Since there are previous records on the presence/ induction of antifungal phenolics in plants following biotic stresses, in the present study such phenols were extracted from tea leaves subjected to biotic stress, temperature stress. Further, since different stresses may induce similar response such antifungal phenolics were also extracted from tea leaves subjected to abiotic stress i.e. elevated temperature. The phenolics were bioassayed and were also analysed UV-spectrophotometrically.

4.7.2.1. Bioassay

The antifungal phenolics were extracted following procedure mentioned in the materials and methods. All the four fractions obtained were bioassayed by petriplate method. Out of the four fractions only fraction III (ethyl acetate fraction) showed antifungal property and hence further TLC plate bioassay was done with this fraction III alone (Plate XIII).

4.7.2.1.1. Elevated temperature

Presence of antifungal phenolics was detected in control as well as temperature stresses leaves by both petriplate and TLC plate bioassays. However,no differences in the nature of inhibition zone was apparent in the petriplate bioassays. In TLC plate bioassay the inhibition zone was apparent at Rf (0.66). In all cases, the diameter of the inhibition zone in control leaves is slightly higher than the treated leaves (Plate XIV). No differences could however be detected among the different temperature treatments.

4.7.1.2.2. Biotic stress

In petriplate bioassay the extracts of healthy leaves showed greater inhibition than those from infected leaves (Plates XV). Same trend was also observed in TLC plate bioassays where the diameter of the inhibition zone was greater in the healthy leaf extracts than in the infected ones (Plate XVI). The Rf of the inhibition zone in this case was also similar to that observed in the temperature treatment.

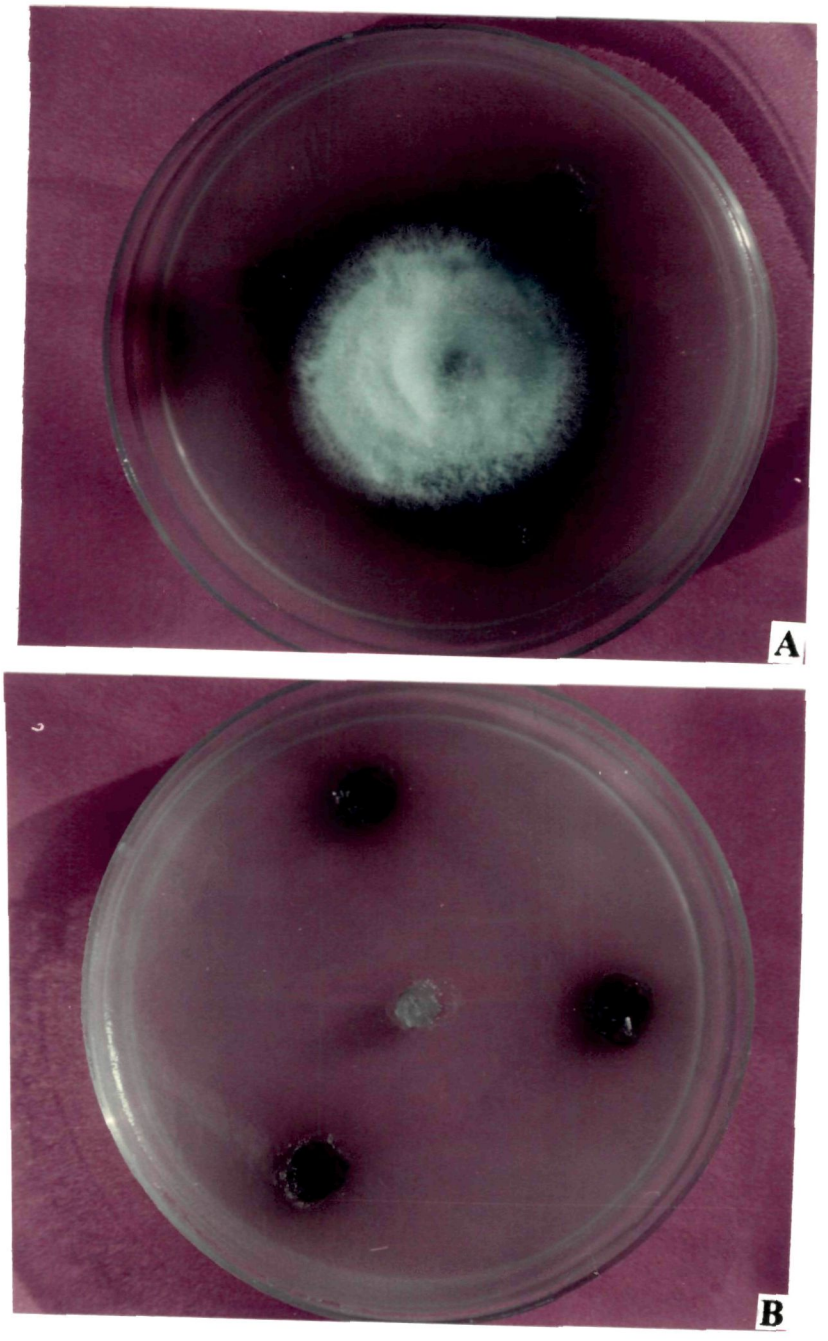


Plate XIII (figs. A&B) : Petriplate bioassay of antifungal phenolics from healthy tea leaves (UP-3); A- Fraction II showing no inhibition; B - Fraction II showing complete inhibition

4.7.2.2. UV-Spectrophotometric analysis

Results of the bioassay revealed the presence of antifungal compounds in all cases i.e. healthy, elevated temperature stresses, as well as blister blight infected tea leaves. On the TLC plates, the Rf values of all these compounds were same. In order to determine whether differences exist in their UV-absorption spectra, these were then analysed by UV- spectrophotometer.

4.7.2.2.1. Elevated temperatures

The UV – spectra from all the elevated temperature treatments had a sharp peak at 221 nm. In the control extracts, the peak at 221 nm was present, but not very sharp; but one at 272 nm was more prominent. This peak was also present at elevated temperatures, but their height was much smaller. Other small humps and shoulders were also evident in all cases (Figs. 20 and 21). Results revealed that the nature of phenolics varied with the stress, even though their Rf in TLC plate bioassay was same.

4.7.2.2.2. Biotic stress

In case of blister infected leaf also, the peak at 221 nnm was very sharp, with no other sharp peak (Fig. 22).

1 2 3 4

Plate XIV : TLC plate bioassay of antifungal phenolics from leaves of tea plants (UP-3) subjected to temperature stress. Lane 1- Control, 2- 40^{0}C, 3-45^{0}C & 4- 45^{0}C

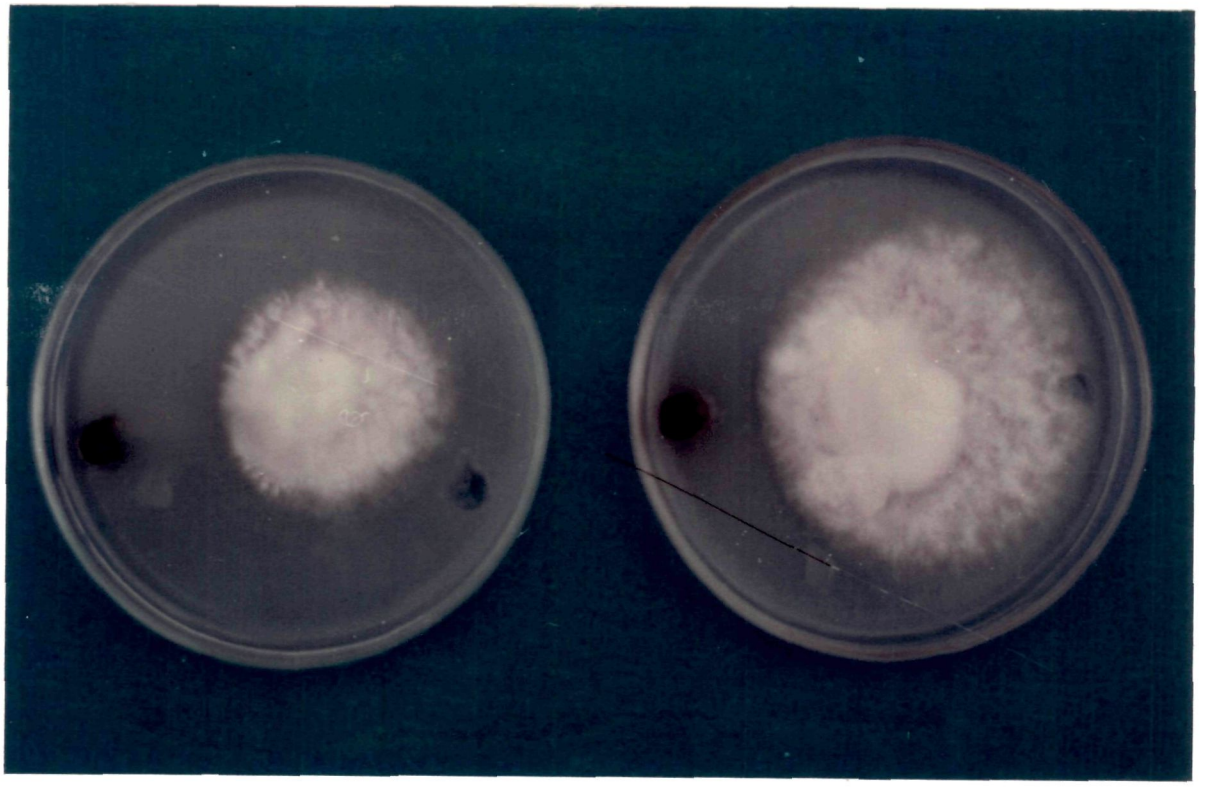
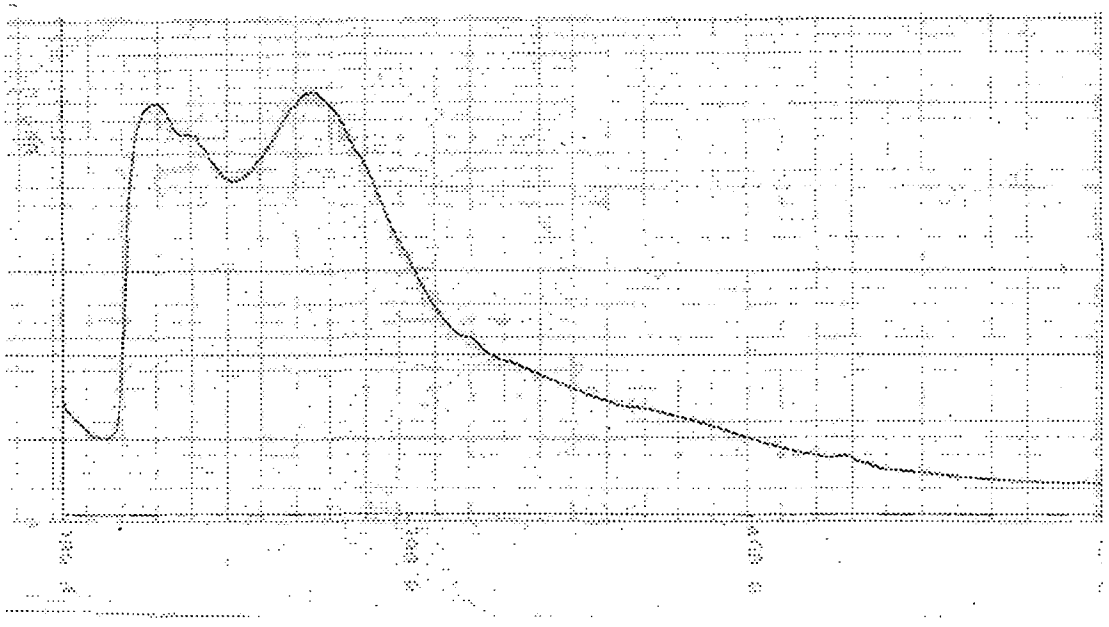


Plate XV : Petri plate bioassay of antifungal phenolics from leaves of tea plants subjected to biotic stress. Left – Healthy ; Right-Blister blight infected

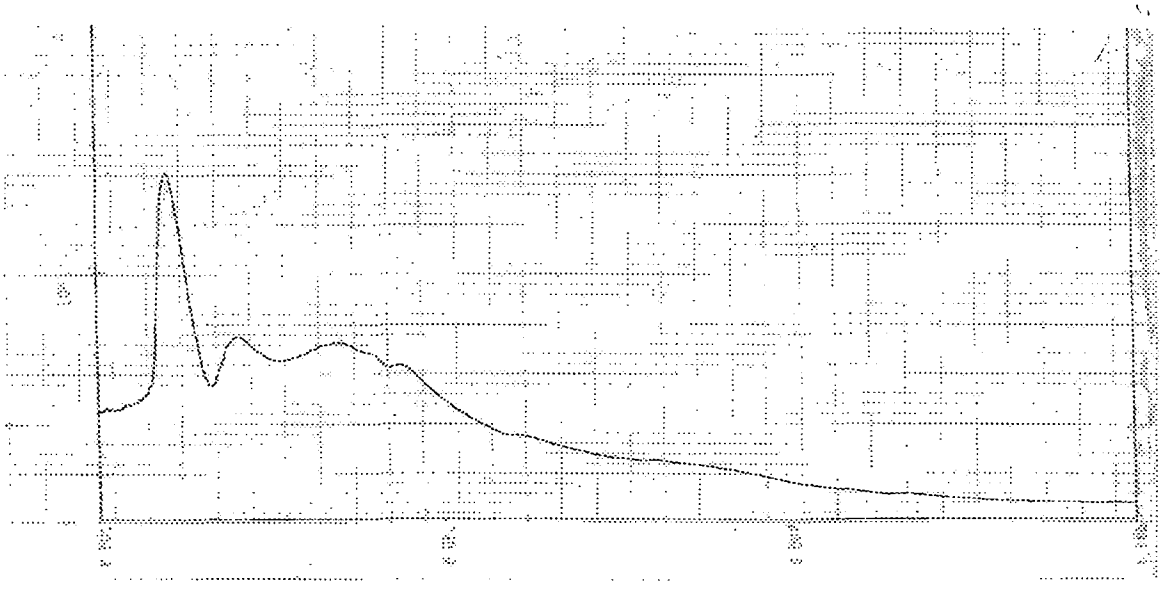
1 2

Plate XV : TLC plate bioassay of antifungal phenolics from leaves of tea plants subjected to biotic stress. Lane 1- Healthy; 2- Blister blight infected

Changes of antifungal phenolics of tea plants subjected to elevated temperatures



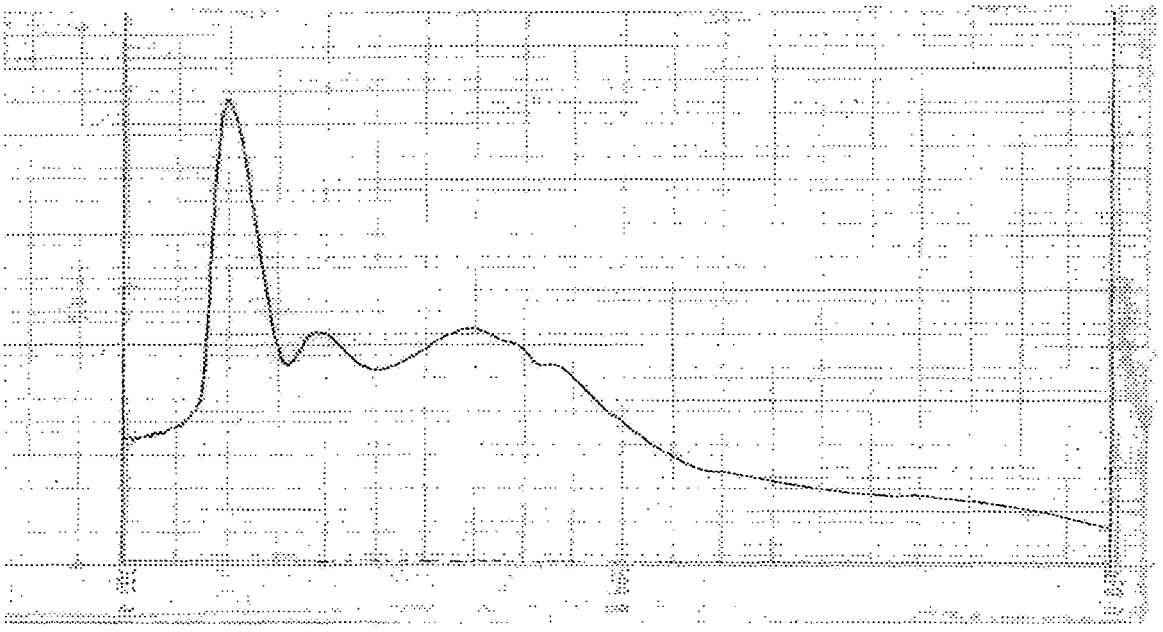
Control



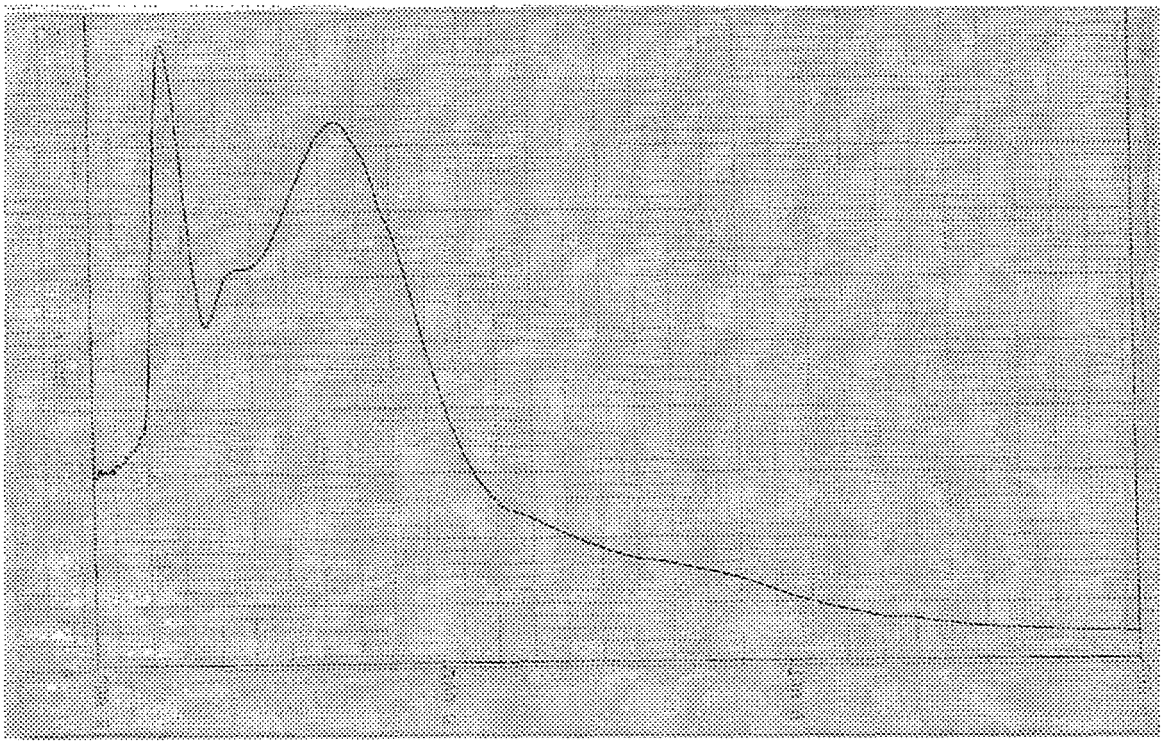
40^{0}C temperature elevation

Fig. 20

UV-spectrophotometric analysis of antifungal phenolics of tea plants subjected to elevated temperatures



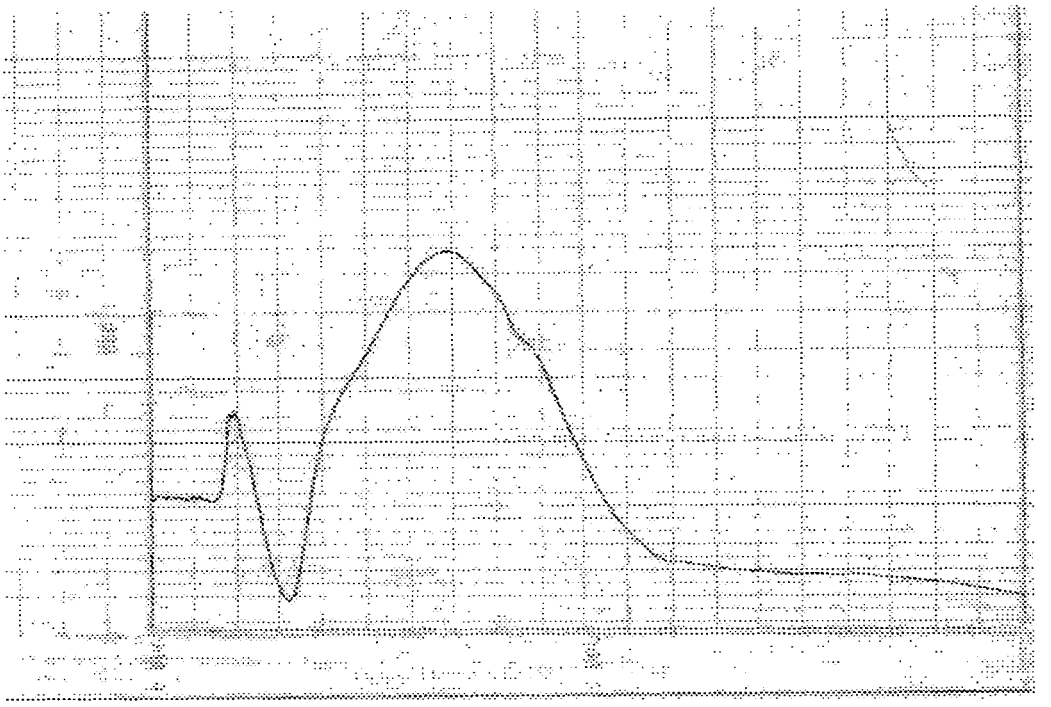
45^{0}C temperature elevation



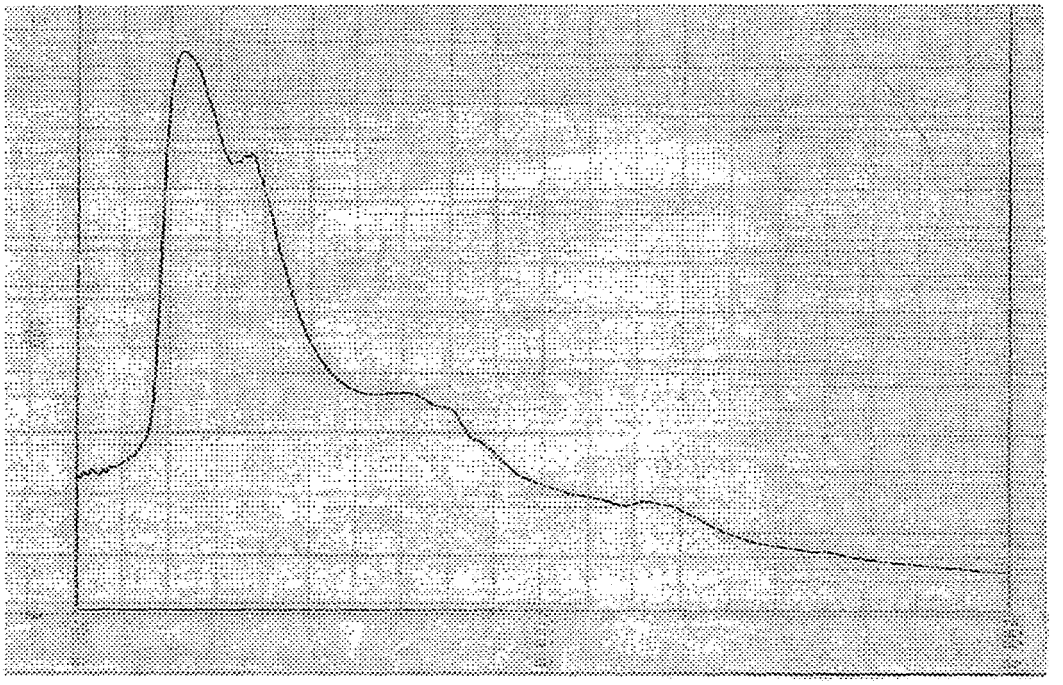
50^{0}C temperature elevation

Fig. 21

Changes of antifungal phenolics of tea plants following Blister Blight infection



Healthy leaves



Blister infected leaves

Fig. 22

4.7.3. HPLC analysis of catechins

HPLC analysis of catechins from healthy and blister infected leaves revealed that no major changes in the types of catechins .However, two major peaks showed lesser peak height in the infected extract, in comparison to the healthy ones(Tables 51 and 52, Fig. 23).

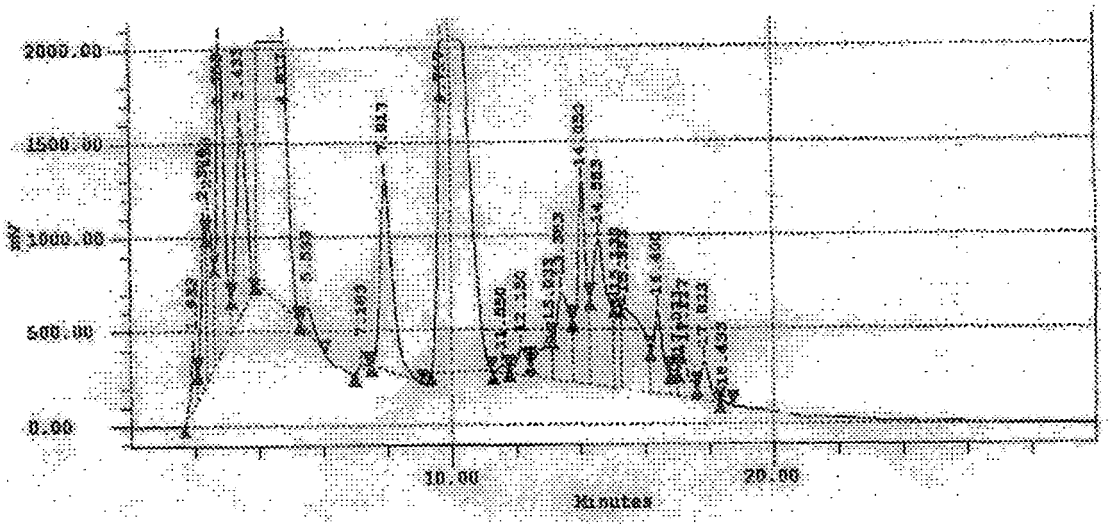
Table 51: Peak results of HPLC analysis of catechin extracts of healthy tea leaves

No. of peaks	Ret time (min.)	Area (UV* sec)	Height (uv)	Int type	% Area	% Height
1.	1.933	3577209	289373	BV	0.82	2.10
2.	2.300	9972866	858334	VV	2.30	6.21
3.	2.433	11092668	876050	VV	2.55	6.34
4.	2.783	28555251	1679102	VV	6.55	12.16
5.	3.433	23507692	1020213	VB	5.41	7.39
6.	4.871	84312080	1421551	BM	19.42	10.29
7.	5.533	2949636	132650	BM	0.66	0.96
8.	7.183	533608	40243	MM	0.12	0.29
9.	7.917	27288806	1015024	VB	6.29	7.35
10.	9.717	118633727	1773628	MM	27.33	12.84
11.	11.550	885836	47476	BM	0.20	0.34
12.	12.150	3744384	151632	MM	0.86	1.10
13.	13.033	7060335	233981	MM	1.63	1.69
14.	13.383	14250383	477555	MM	3.28	3.46
15.	14.050	21824594	1071982	MM	5.03	7.76
16.	14.583	27101987	790583	MM	6.24	5.72
17.	15.133	6111840	446103	MM	1.41	3.23
18.	15.383	20885483	436255	MM	4.81	3.16
19.	16.400	9029354	449911	MM	2.26	3.26
20.	17.033	2427316	149819	MM	0.56	1.08
21.	17.217	4987499	217138	MM	1.15	1.57
22.	17.833	4451310	213598	MM	1.03	1.55
23.	18.433	273936	20022	MM	0.06	0.14

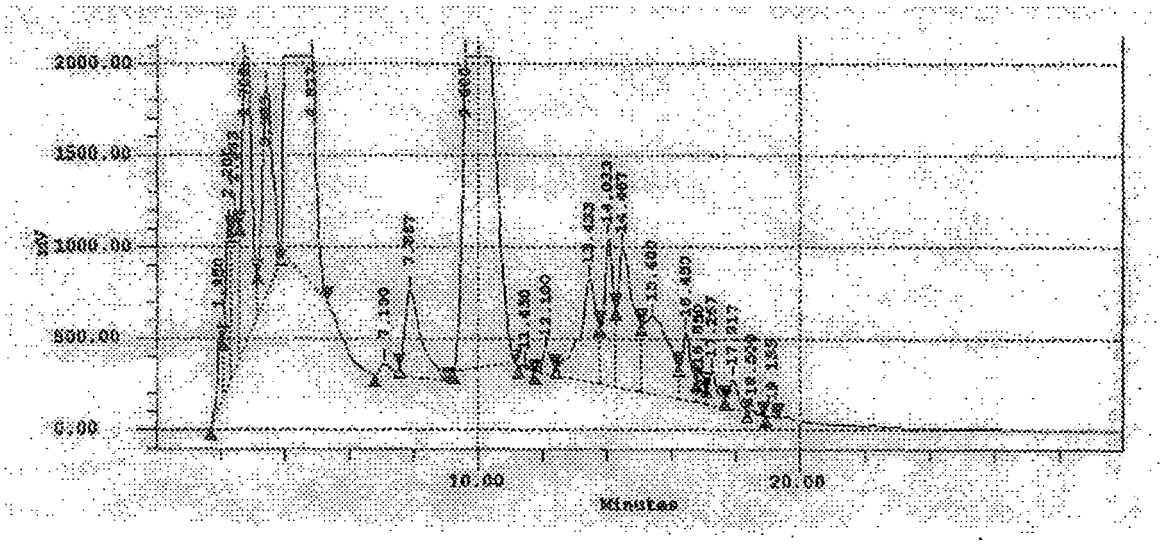
Table 52: Peak results of HPLC analysis of catechin extracts of blister infected tea leaves

No. of peaks	Ret time (min.)	Area (UV* sec)	Height (uv)	Int type	% Area	% Height
1.	1.950	4597962	403144	BV	1.09	3.31
2.	2.200	10829212	903547	VV	2.57	7.43
3.	2.383	12545341	909876	VV	2.98	7.48
4.	2.700	34488798	1607982	VV	8.18	13.22
5.	3.383	26082251	1156801	VB	6.19	9.51
6.	4.871	76264259	1254742	BM	18.09	10.32
7.	7.100	1941800	68940	BV	0.46	0.57
8.	7.867	17499917	477256	VB	4.15	3.92
9.	9.600	123139285	1720155	MM	29.21	14.14
10.	11.450	372342	23605	BB	0.09	0.19
11.	12.100	2949148	136867	BM	0.70	1.13
12.	13.433	22383609	568617	MM	5.31	4.67
13.	14.033	17437223	814879	MM	4.14	6.70
14.	14.467	27410731	778076	MM	6.50	6.40
15.	15.400	25240830	436719	MM	5.99	3.59
16.	16.450	8042319	379674	MM	1.91	3.12
17.	16.850	2368535	129706	MM	0.55	1.07
18.	17.267	3887023	194292	MM	0.92	1.60
19.	17.917	3669391	167295	MM	0.87	1.38
20.	18.500	354944	24125	MM	0.80	0.20
21.	19.133	81661	6780	BV	0.02	0.06

Changes in HPLC profile of catechin of tea plants following Blister Blight infection



Healthy leaves



Blister infected leaves

Fig. 23