



Results and Discussion

4. RESULTS AND DISCUSSION

The results of the study are discussed under the following headings.

- 4.1. Isolation of *Bacillus Subtilis* from Textile Waste Effluent**
- 4.2. Isolation of *Thiobacillus Bacteria* from Textile Waste Effluent**
- 4.3. Decolorization of textile dye effluent using *Bacillus Subtilis***
- 4.4. Analysis of Odor Degradation**
- 4.5. Analysis of effluent and decolorized effluent water**
- 4.6. Evaluation of the dyed sample**

4.1. ISOLATION OF *BACILLUS SUBTILIS* FROM TEXTILE WASTE EFFLUENT

The result of the biochemical test for the isolation of *Bacillus subtilis* from Textile waste effluent is given in Table IV.

TABLE IV
ISOLATION OF *BACILLUS SUBTILIS* FROM TEXTILE WASTE EFFLUENT

S.No	Biochemical test	Result
1	Catalase test	+
2	Oxidase test	d
3	Urease test	-
4	Indole test	-
5	Methyl Red test	-
6	Voges proskauer test	+
7	Citrate utilization test	+
8	Starch hydrolysis test	+
9	Gelatin hydrolysis test	+
10	Caesin hydrolysis test	+
11	Nitrate reduction test	+
12	Sugar fermentation test D-Glucose	+
13	L-Arabinose	+
14	D-Mannitol	+
15	D-xylose	+
16	Gas from Glucose	-

From the above Table IV, it is evident that the biochemical test such as Catalase test, Voges proskauer test, Citrate utilization test, Starch hydrolysis test, Gelatin hydrolysis test, Caesin hydrolysis test, Nitrate reduction test, Sugar fermentation test D-Glucose, L-Arabinose, D-Mannitol and D-xylose showed positive result and Urease test, Indole test, Methyl Red test and Gas from Glucose showed negative result, Oxidase test showed result as d, which means variable (may be positive or negative).

4.2. ISOLATION OF *THIOBACILLUS BACTERIA* FROM TEXTILE WASTE EFFLUENT

The result of the biochemical test for the isolation of *Thiobacillus bacteria* from textile waste effluent is given in Table V.

TABLE V
ISOLATION OF *THIOBACILLUS BACTERIA* FROM TEXTILE WASTE EFFLUENT

S.No	Biochemical test	Result
1	Catalase test	+
2	Oxidase test	-
3	Urease test	+
4	Indole test	-
5	Methyl Red test	+
6.	TSI	A/A, Gas,
7.	Voges proskauer test	-
8.	Citrate utilization test	+
9.	Sugar fermentation test D-Glucose	+
10.	L-Arabinose	+
11.	D-Mannitol	+
12.	D-xylose	+

From the above Table V, it is evident that the biochemical test such as Catalase test, Urease test, Methyl Red test, Citrate utilization test, Sugar fermentation test D-Glucose, L-Arabinose, D-Mannitol and D-xylose Showed positive result and Oxidase test, Indole test and Voges proskauer test showed negative result, TSI showed result as A/A, Gas, Acid sland and /acid bud, Gas produced in tube.

4.3. DECOLORIZATION OF TEXTILE DYE EFFLUENT USING *BACILLUS SUBTILIS*

4.3.1. Effect of Inoculum concentration on decolorization of Textile dye Effluent

The effect of inoculum concentration on decolorization was determined and the results are presented in Table VI and Figure I.

TABLE VI
EFFECT OF INOCULUM CONCENTRATION ON DECOLORIZATION

Inoculum concentration (%)	Decolorization (%)
5	1.250
10	1.430
15	1.665
20	1.884
25	1.300
30	1.200

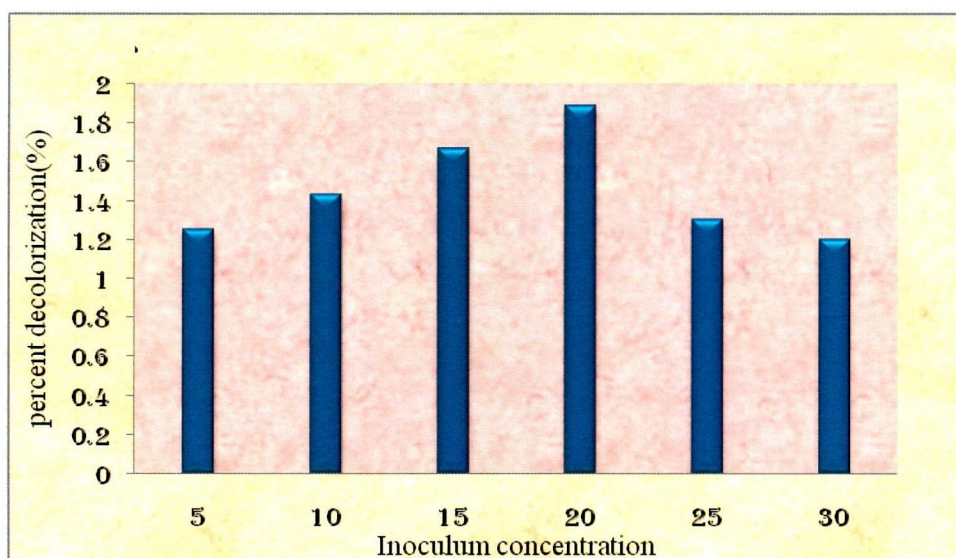


FIGURE I
EFFECT OF INOCULUM CONCENTRATION ON DECOLORIZATION

The increase in inoculum concentration from 5% to 20% showed better decolorization, with optimum decolorization at 3%. However increase in inoculum concentration above 20% resulted in decrease of decolorization. Hence it can be concluded that the optimum inoculation is 20 percent.

4.3.2. Effect of Incubation period on decolorization of Textile dye effluent

The effect of incubation period on decolorization of Textile dye effluent by *Bacillus subtilis* is presented in Table VII and Figure II.

TABLE VII
EFFECT OF INCUBATION PERIOD ON DECOLORIZATION

Days	Decolorization (%)
1	24.3
2	35.0
3	59.64
4	40.3
5	22.8
6	7.01

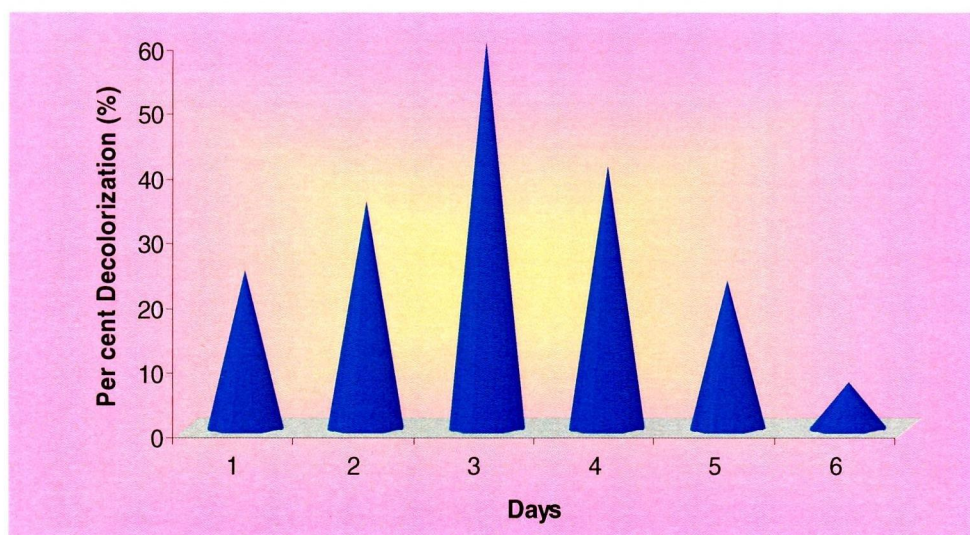


FIGURE II
EFFECT OF INCUBATION PERIOD ON DECOLORIZATION

With reference to the period of decolorization, the effect was observed right from the first day. The maximum decolorization was observed on the third day. Thereafter there was a decrease in decolorization with increase in days.

Hence it can be concluded that third day of the incubation was the optimized incubation period.

4.3.3. Effect of pH on decolorization of Textile dye effluent

The effect of pH on decolorization of Textile dye effluent by *Bacillus Subtilis* is presented in Table VIII and Figure III.

TABLE VIII
EFFECT OF pH ON DECOLORIZATION

S.No	pH	Decolorization rate mg/l/h
1.	5	0.700
2.	5.5	0.805
3.	6.0	1.011
4.	6.5	1.990
5.	7.0	1.240
6.	7.5	1.299

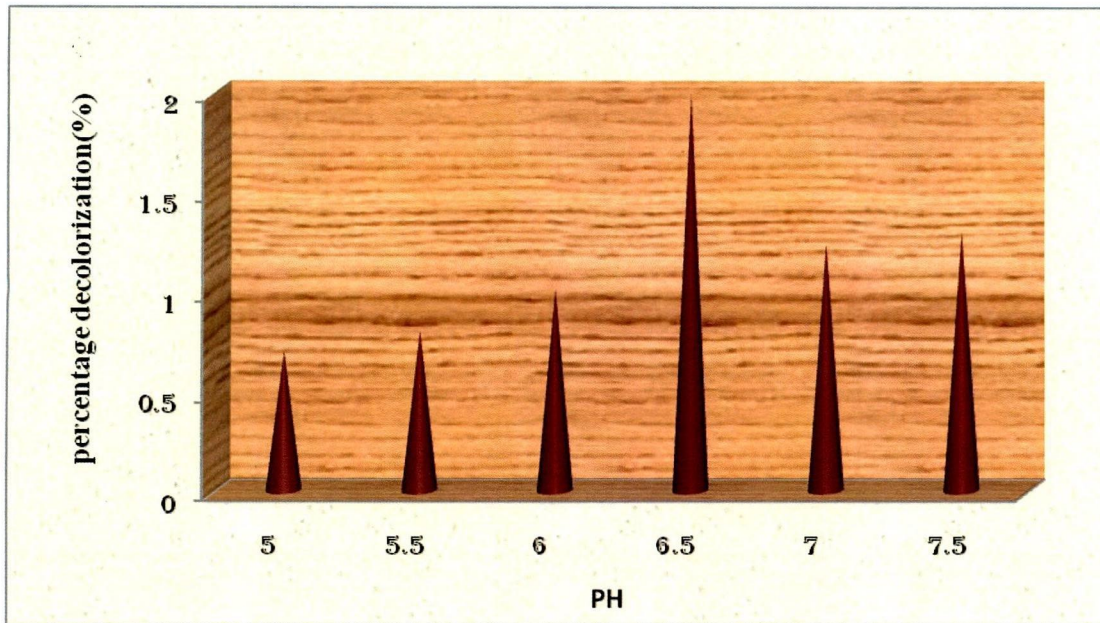


FIGURE III
EFFECT OF pH ON DECOLORIZATION

From the Table VIII and Figure III, it is clear that increase in pH results in increased decolorization of the textile effluent. The maximum decolorization is seen when the pH is 6.5. Thereafter an increase in the pH showed a decrease in decolorization.

Hence it can be concluded that pH 6.5 was the optimized pH. However pH 7 was observed as best by Shanooba *et al.*, (2011) for degradation of textile industrial effluent.

4.3.4. Effect of temperature on decolorization of Textile dye effluent by *Bacillus Subtilis*

The effect of temperature on decolorization of Textile dye effluent by *Bacillus Subtilis* is depicted in Table IX and Figure IV.

TABLE IX
EFFECT OF TEMPERATURE ON DECOLORIZATION

S.No	Temperature (°C)	Decolorization rate mg/l/h
1.	20	0.630
2.	30	1.010
3.	37	1.203
4.	40	1.015
5.	50	0.400

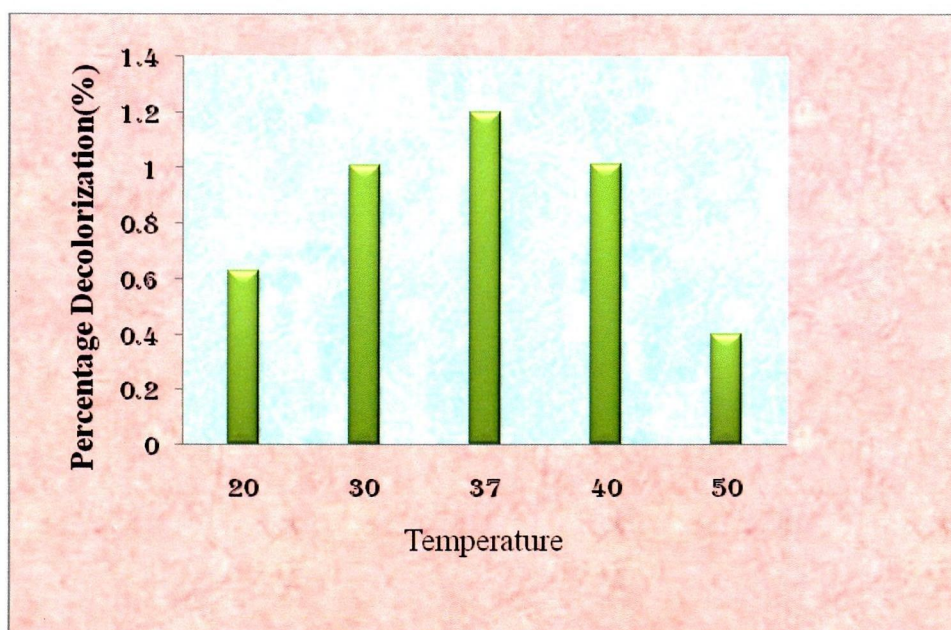


FIGURE IV
EFFECT OF TEMPERATURE ON DECOLORIZATION

Among the different temperatures namely 20°C, 30°C, 37°C, 40°C and 50°C, 37°C was seen as the an optimum temperature for maximum decolorization. There is a decrease in percentage decolourisation after 37°C. Hence it can be concluded that temperature 37°C was the optimized temperature.

Similar results are reported by Shanooba *et al.*, (2011) in their research work.

4.3.5. Effect of Co Substrate on Textile Dye Effluent Decolorization

The effect of co substrates on decolorization of Textile dye effluent by *Bacillus Subtilis* is depicted in Table X and Figure V.

TABLE X

DECOLORIZATION RATE AT DIFFERENT CO SUBSTRATES

S. No	Co substates	% of Decolorization
1.	BHM	11%
2.	BHM +starch + yeast extract	55%
3.	BHM +starch + peptone	90%
4.	BHM +Dextrose +yeast extract	70
5.	BHM+ Sucrose + yeast extract	60
6.	BHM+ Sucrose	40

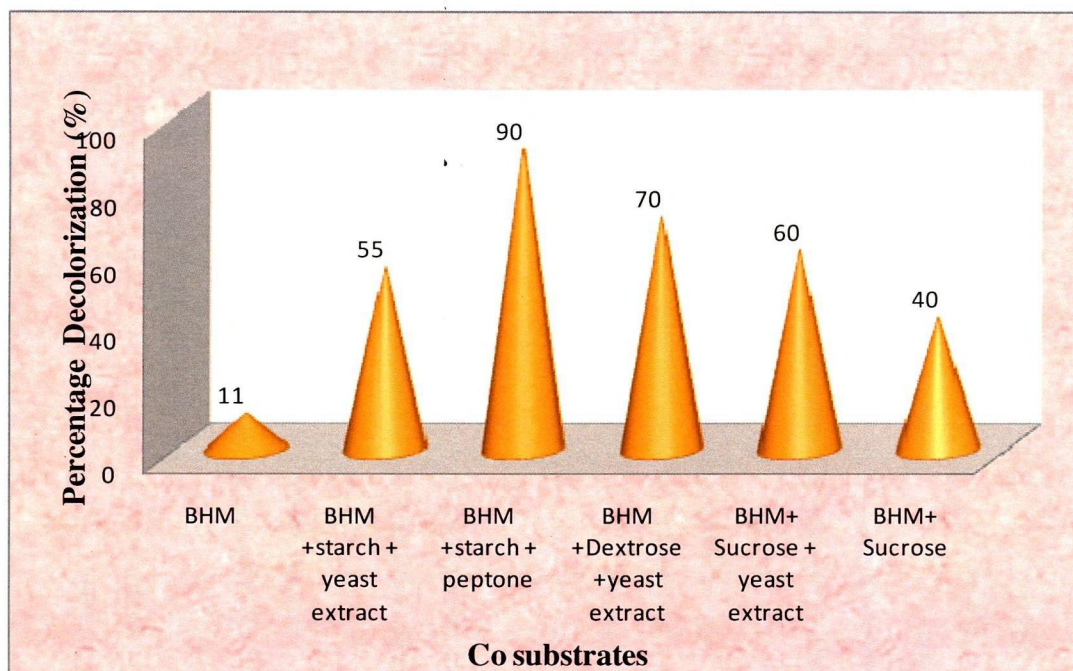


FIGURE V

DECOLORIZATION RATE AT DIFFERENT CO SUBSTRATES

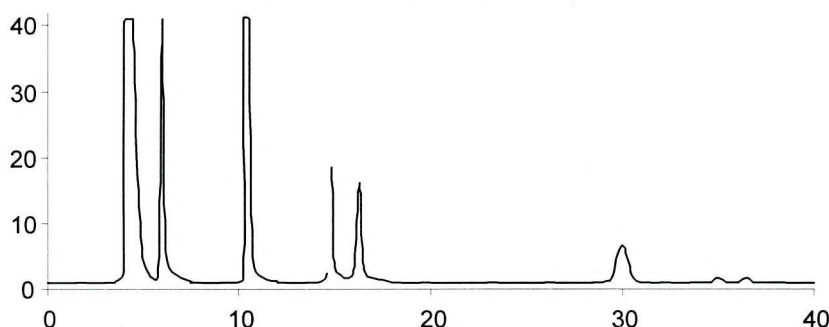
Among the different co substrates namely BHM, BHM +starch + yeast extract, BHM +starch + peptone, BHM +Dextrose +yeast extract, BHM+ Sucrose + yeast extract and BHM+ Sucrose, BHM +starch + peptone was seen as the an optimum co substrates for maximum decolorization. There is a decrease in percentage decolourisation after BHM +starch + peptone.

Hence it can be concluded that inoculation of co substrates BHM +starch + peptone was the optimized co substrates.

4.4. ANALYSIS OF ODOR DEGRADATION

The analysis of odor from the textile based effluent is shown in Graph 1 and 2 (Appendix III)

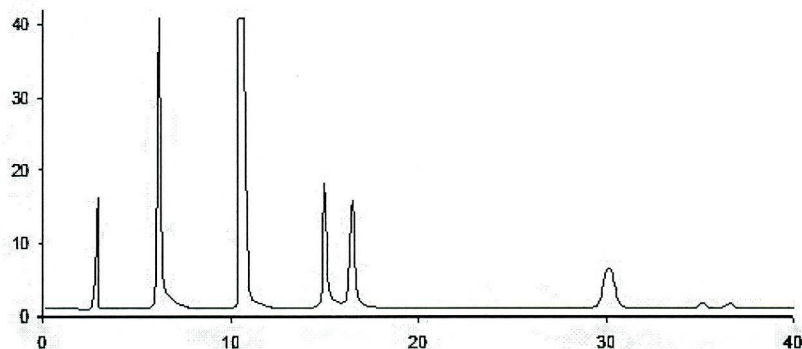
**Graph - 1
Control (without Bacteria)**



1. H ₂ S => 0.066746	5. DMS=>0.001927
2. Cos => 0.00652	6. DMD=>0.001416
3. MesH => 0.022413	7. MEDS=>0.000766
4. SO ₂ => 0.02164	8. DEDES=> 0.005031

**Graph-2
Test Sample (with Bacteria)**

Hydrogen reduced. Two other compounds detected.



1. H ₂ S => 0.001830	5. DMS=>0.001927
2. Cos => 0.00631	6. DMD=>0.001416
3. MesH => 0.022312	7. MEDS=>0.000766
4. SO ₂ => 0.02164	8. DEDES=> 0.005031

From the Graphs it is clear that after introducing *Thiobacillus Bacteria* the hydrogen sulphide level has decreased by 36 per cent proving reduction in odor. Hence it could be concluded that odor can be removed by *Thiobacillus Bacteria*.

4.5. ANALYSIS OF EFFLUENT AND DECOLORIZED EFFLUENT WATER

TABLE XI

PHYSICOCHEMICAL PARAMETERS OF EFFLUENT SAMPLES

S.No	Parameters	TWE	TWB	TNPCB
1.	Color (285nm)	57	0.027	
2.	pH	8.5	7.5	5.5 – 9
3.	Electrical conductivity (Ω)	4350	512	-
4.	Turbidity (NTU)	6.8	2.10	-
5.	Total suspended solids (mg/l)	1620	85	100
6.	Total dissolved solids (mg/l)	2670	720	2100
7.	Biological oxygen demand	160	90	30
8.	Chemical oxygen demand		-	
9.	Total hardness (mg/l)	720	48	600
10.	Phenolphthalein alkalinity (as CaCO_3) (mg/l)	42	28	-
11.	Total alkalinity (as CaCO_3) (mg/l)	4200	15.6	-
12.	Calcium (mg/l)	440	0.06	-
13.	Phosphate (mg/l)	0.08	-	-
14.	Sulphate (mg/l)	200		-

TWE – Textile Waste Effluent

TNPCB – TamilNadu pollution control board

TWB – Treated Water by Bacteria

From the water analysis, it is obvious that the decolorization efficiency was excellent after effluent treatment with *Bacillus Subtilis*.

Turbidity level of the effluent was 6.8 NTU and it was reduced to two after treatment using *Bacillus Subtilis Bacteria*.

Total dissolved solids of the effluent was 2670 mg/l. It has been reduced to 720 mg/l and 640 mg/l by *Bacillus Subtilis Bacteria* respectively and the levels are within the range recommended by TNPCB.

TSS also reduced from 1620 mg/l to 85 mg/l and 86 mg/l by *Bacillus Subtilis Bacteria*.

Similarly total hardness was reduced from 720 mg/l to 48 mg/l and 47 mg/l by *Bacillus Subtilis Bacteria* and the levels are within the range recommended by TNPCB.

Phenolphthalein alkalinity was totally removed from 42 mg/l by *Bacillus Subtilis Bacteria*.

Total alkalinity of the effluent was reduced by the treatment very effectively.

BOD was reduced considerably after treatment, but it is higher than the range recommended by TNPCB norms, this is due to the presence of organic matter in treated water.

All the other components are reduced considerably after treatment *Bacillus Subtilis Bacteria*.

4.6. EVALUATION OF THE DYED SAMPLE

4.6.1. Subjective Evaluation

4.6.1.1. Visual Inspection of the Dyed Sample

Subjective evaluation of the samples SWD and TWD are presented in Table XII.

TABLE XII**SUBJECTIVE EVALUATION**

S. No	Sample	Presented in percentage						Evenness of dyeing	
		General appearance			Brightness of shade				
		Excellent	Good	Fair	Bright	Medium	Dull	Even	Uneven
1.	SWD	40	60	-	80	20	-	100	-
2.	TWD	35	65	-	80	20	-	100	-

From Table XII it is evident that the sample dyed with effluent treated water (TWD) scored 65 percent and ranked good for general appearance than the samples dyed using soft water. With regard to brightness 80% of judges rated TWD, sample as bright. Both the samples were rated to be evenly dyed as expressed by all the judges. Therefore it is clear that the treated effluent water could be used for dyeing.

4.6.2. Objective Evaluation**4.6.2.1. Fabric Weight**

The Fabric weight and analysis of variance of the samples original, SWD and TWD are given in Table XIII and Figure VI.

TABLE XIII

FABRIC WEIGHT

S.No	Samples	Mean wt in (GSM)	Loss / gain over original	% loss / gain over original
1.	Original	1.203	-	-
2.	SWD	1.208	-0.005	0.41
3.	TWD	1.207	0.004	0.33

Anova for Fabric Weight % Loss or Gain

	Sum of Squares	Degree of freedom	Mean Square	F
Between Groups	0.000026	2	0.000061	18.8866**
Within Groups	0.000149	14	0.000011	
Total	0.000175	16	0.000072	

** Significant at 1% level

From Table XIII and Figure VI it is clear that the weight of samples increased after dyeing when compared to the original. The percentage weight increase in SWD sample was 0.41% and TWD sample was 0.33% respectively.

Statistical analysis proves that there is a significant difference at 1% level between original and dyed samples. Maximum increase in sample TWD in weight may be the result of more dye absorption. The results prove that the treated waters can be effectively used for dyeing cotton and also increase in weight in fabric TWD is similar with that of SWD.

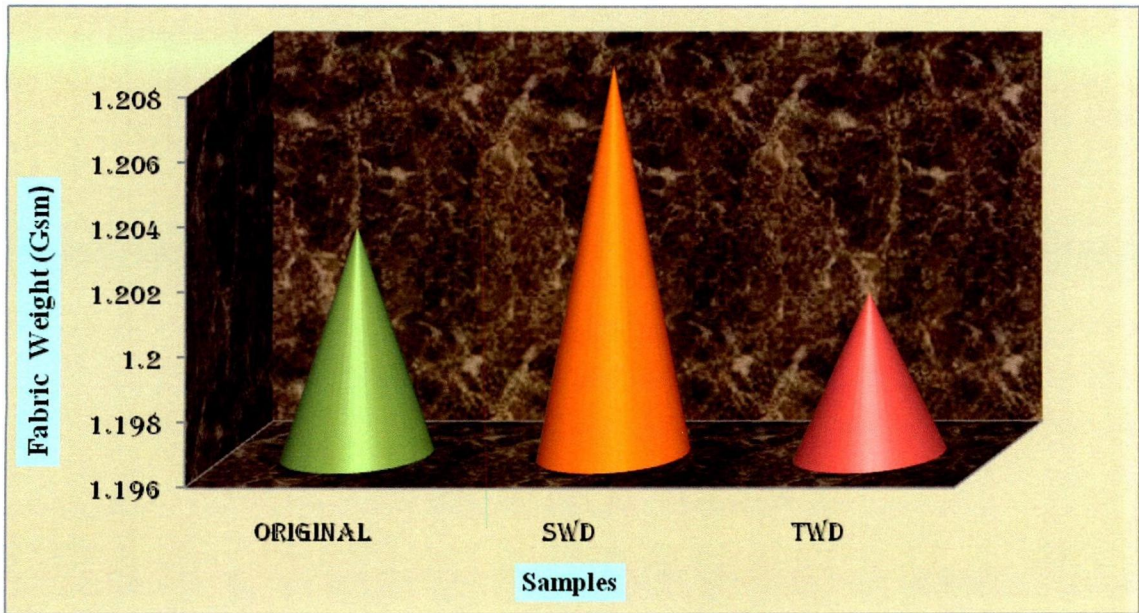


FIGURE VI
FABRIC WEIGHT

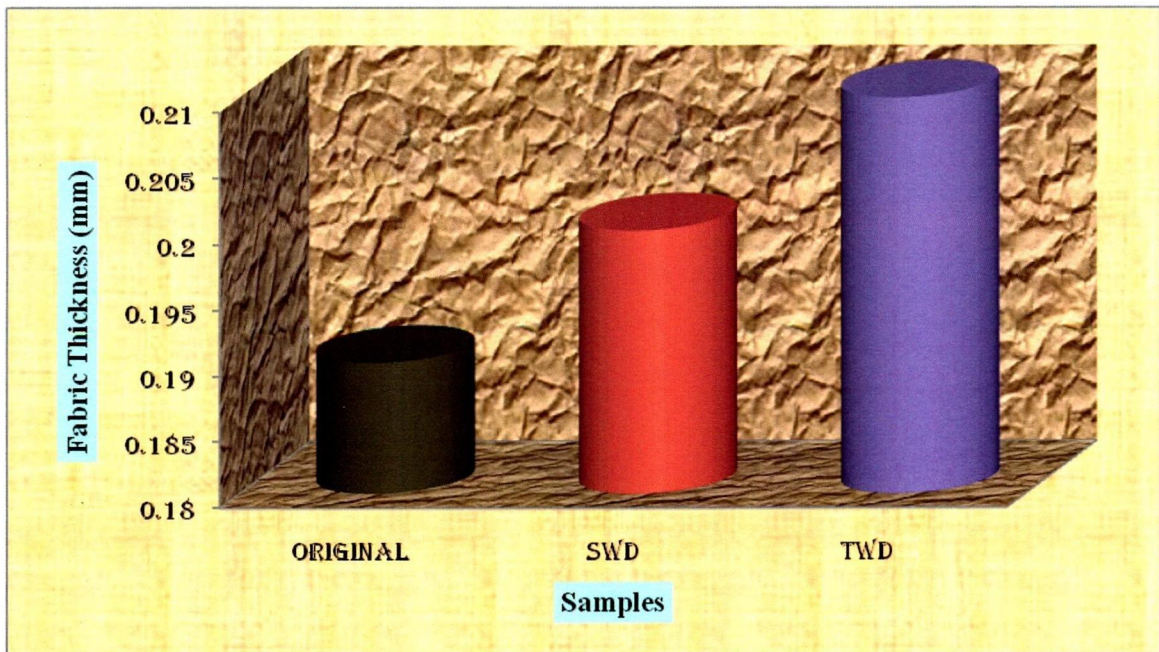


FIGURE VII
FABRIC THICKNESS

4.6.2.2. Fabric Thickness

Thickness and analysis of variance of the samples O, SWD and TWD are presented in the Table XIV and Figure VII.

TABLE XIV
FABRIC THICKNESS

S.No	Samples	Mean wt in thickness (mm)	Loss / gain over original	% loss / gain over original
1.	Original	0.19	-	-
2.	SWD	0.20	-0.01	5.26
3.	TWD	0.21	-0.02	10.52

Anova for Fabric Thickness % Loss or Gain

	Sum of Squares	Degree of freedom	Mean Square	F
Between Groups	0.00108	3	0.00036	4.80*
Within Groups	0.00228	19	0.000075	
Total	0.00336	22	0.000435	

*Significant at 5% level

From the Table XIV and Figure VII it is evident that the thickness of the dyed samples increased when compared to the original. The per cent increase in SWD was five per cent and TWD was ten per cent over original. Statistical analysis proves that there is a significant difference at 5% level between original and dyed samples.

Thus it could be concluded that maximum increase in thickness may be the result of more dye absorption by sample TWD and the result also proves the concept of using effluent water treated with *Bacillus subtilis* for effective reactive dyeing on cotton.

4.6.2.3. Fabric stiffness

Fabric stiffness and analysis of variance of the samples original, SWD and TWD in warp direction is presented in Table XV and Figure VIII.

TABLE XV

FABRIC STIFFNESS (WARP)

S. No	Samples	Mean wt in (GSM)	Loss / gain over original	% loss / gain over original
1.	Original	2.19	-	-
2.	SWD	2.41	0.22	10.04
3.	TWD	2.24	0.05	2.28

Anova for Fabric Stiffness (Warp) % Loss or Gain

	Sum of Squares	Degree of freedom	Mean Square	F
Between Groups	0.111373	2	0.055687	710.8936**
Within Groups	0.114173	14	0.008155	
Total	0.225546	16	0.063842	

**Significant at 1% level

From Table XV and Figure VIII, it is clear that the fabric stiffness of sample dyed in soft water has increased by 10.04% against original and with

regard to TWD there was 2.28% decrease in stiffness over original which is negligible.

Statistical analysis proves that there is a significant difference at 1% level between original and dyed samples along warp direction.

Fabric stiffness (Weft)

Fabric stiffness and analysis of variance of the samples original, SWD and TWD in weft direction is presented in Table XVI and Figure IX.

TABLE XVI
FABRIC STIFFNESS (WEFT)

S.No	Samples	Mean wt in (GSM)	Loss / gain over original	% loss / gain over original
1.	Original	2.09	-	-
2.	SWD	1.90	-0.19	9.09
3.	TWD	1.85	-0.24	11.4

Anova for Fabric Stiffness (Weft) % Loss or Gain

	Sum of Squares	Degree of freedom	Mean Square	F
Between Groups	0.157493	2	0.078747	1098.7907**
Within Groups	0.159133	14	0.011367	
Total	0.225546	16	0.063842	

** Significant at 1% level

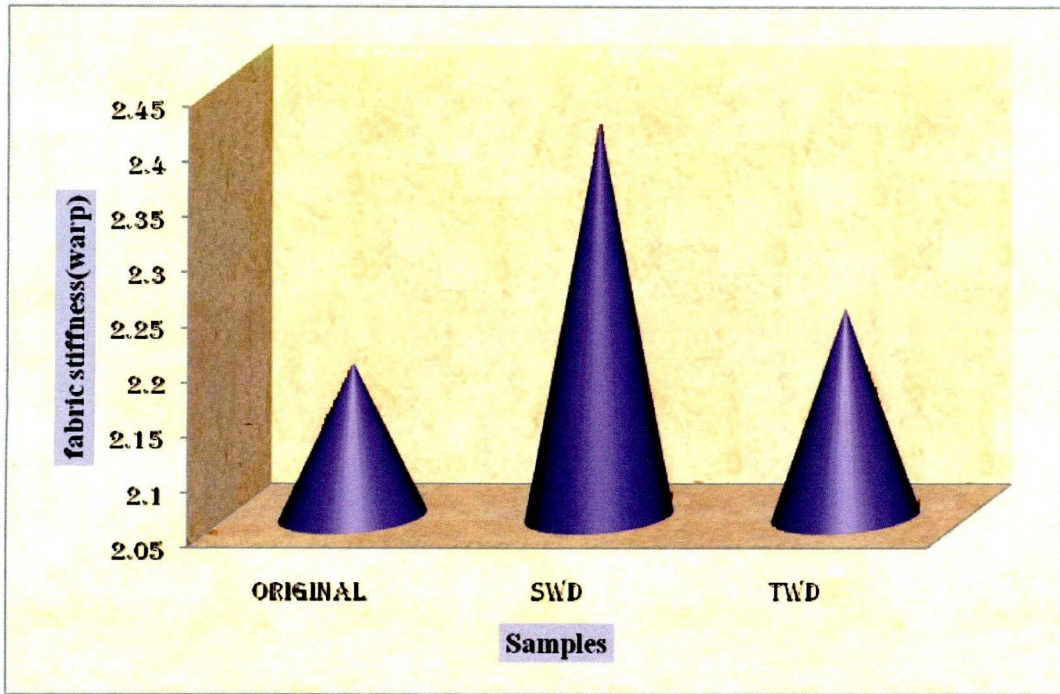


FIGURE VIII

FABRIC STIFFNESS (WARP)

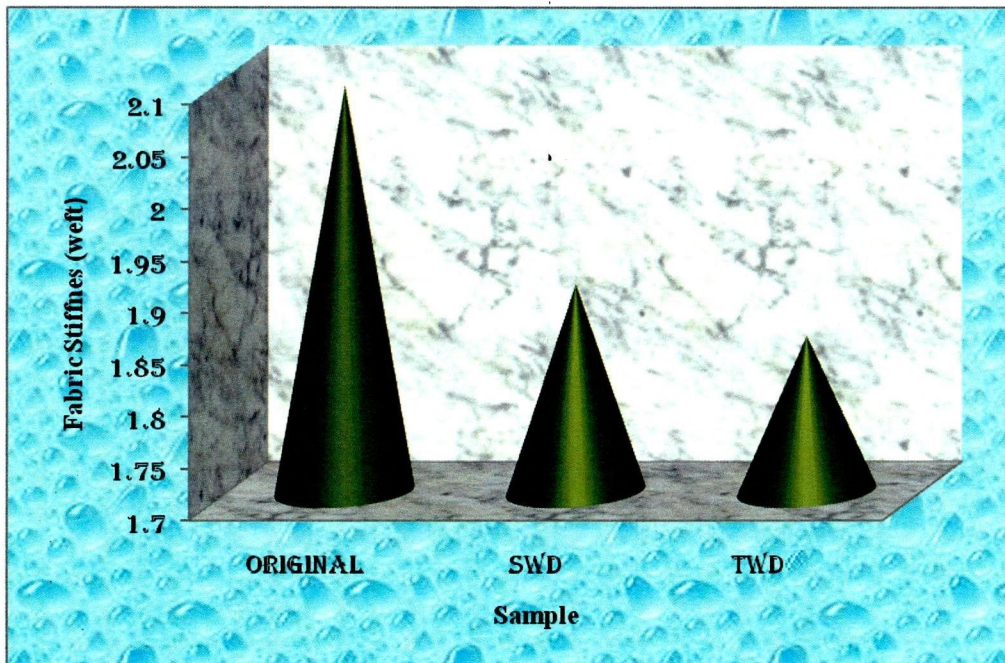


FIGURE IX

FABRIC STIFFNESS (WEFT)

The stiffness of all the samples decreased after dyeing along weft direction. The soft water dyed sample decreased by 9.09% in stiffness over original. With regard to TWD sample the reduction was 11.4% over original.

Statistical analysis proves that there is a significant difference at 1% level between original and dyed samples along weft direction.

Hence from the result it could be concluded that treated water can be utilized for dyeing effectively.

4.6.2.4. Fabric strength (warp)

Tensile strength in warp and analysis of variance direction of the samples O, SWD and TWD are depicted in Table XVII and Figure X.

TABLE XVII
FABRIC STRENGTH (WARP)

S.No	Samples	Mean wt in (GSM)	Loss / gain over original	% loss / gain over original
1.	Original	57	-	-
2.	SWD	61	4	7.01
3.	TWD	62	5	8.77

Anova for Fabric Strength (Warp) % Loss or Gain

	Sum of Squares	Degree of freedom	Mean Square	F
Between Groups	89.733333	2	44.866667	29.2609**
Within Groups	109.333333	14	7.809524	
Total	199.06666	16	52.676191	

**Significant at 1% level

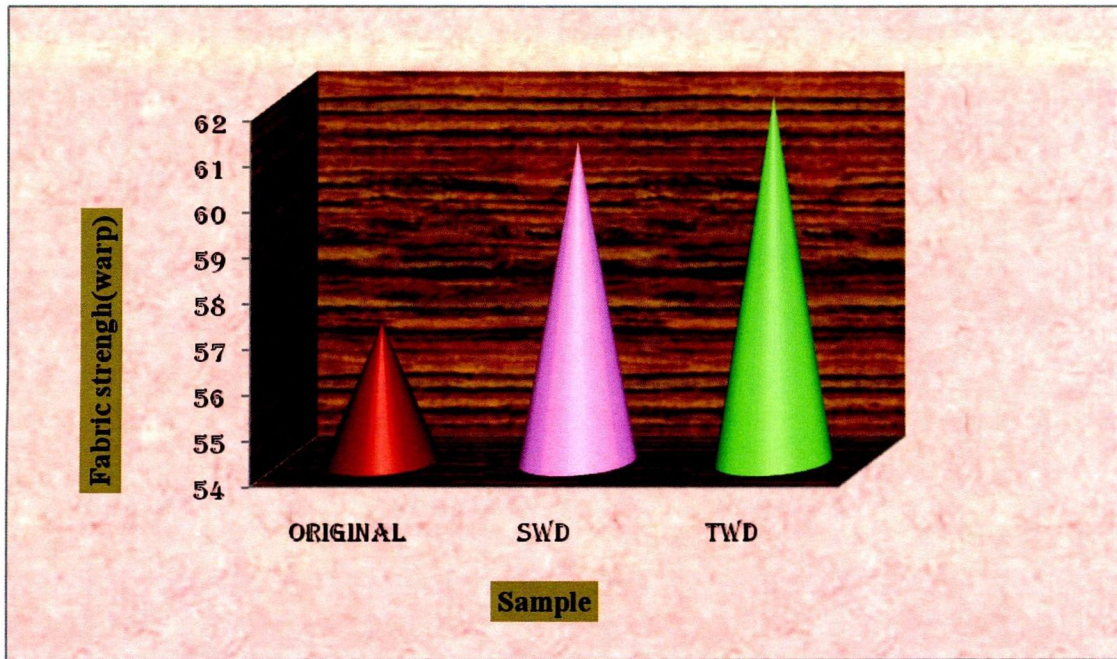


FIGURE X
FABRIC STRENGTH (WARP)

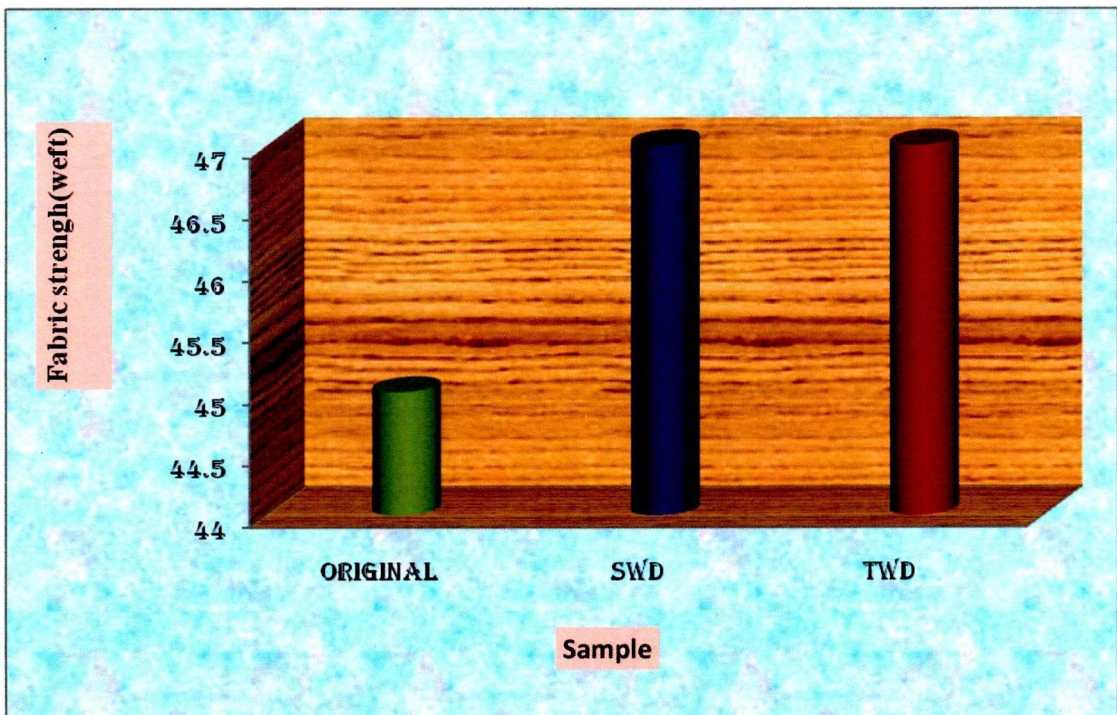


FIGURE XI
FABRIC STRENGTH (WEFT)

From the Table XVII and Figure X it is evident that the strength of the dyed samples in warp direction increased when compared to the original. The percent increase in SWD and TWD are 7% and 8.8% respectively over original.

Statistical analysis proves that there is a significant difference at 1% level between original and dyed samples.

Fabric strength (weft)

Tensile strength and analysis of variance in weft direction of the samples O, SWD, and TWD are depicted in Table XVIII and Figure XI.

TABLE XVIII
FABRIC STRENGTH (WEFT)

S.No	Samples	Mean wt in (GSM)	Loss / gain over original	% loss / gain over original
1.	Original	45	-	-
2.	SWD	47	2	4.4
3.	TWD	47	2	4.4

Anova for Fabric Strength (Weft) % Loss or Gain

	Sum of Squares	Degree of freedom	Mean Square	F
Between Groups	20.800000	2	10.400000	12.7347**
Within Groups	31.600000	14	2.257143	
Total	52.400000	16	12.657143	

** Significant at 1% level

From the Table XVIII and Figure XI it is evident that the strength of the dyed samples in weft direction increased when compared to the original. The per cent increase was 4.4% in both the samples. This proves that the weft direction strength is unaffected by the type of water used for dyeing.

Statistical analysis proves that there is a significant difference at 1% level between original and dyed samples. Hence from the result it could be concluded that treated water can be utilized for dyeing effectively.

4.6.2.5. Fabric Elongation (warp)

Elongation and analysis of variance of the samples O, SWD, and TWD in warp direction are presented in Table XIX and Figure XII.

TABLE XIX
FABRIC ELONGATION (WARP)

S. No	Samples	Mean wt in (GSM)	Loss / gain over original	% loss / gain over original
1.	Original	0.67	-	-
2.	SWD	0.90	0.23	34.3
3.	TWD	0.86	0.19	28.3

Anova for Fabric Elongation (warp) % Loss or Gain

	Sum of Squares	Degree of freedom	Mean Square	F
Between Groups	0.149560	2	0.074780	1043.4419**
Within Groups	0.150560	14	0.010754	
Total	0.300120	16	0.085534	

** Significant at 1% level

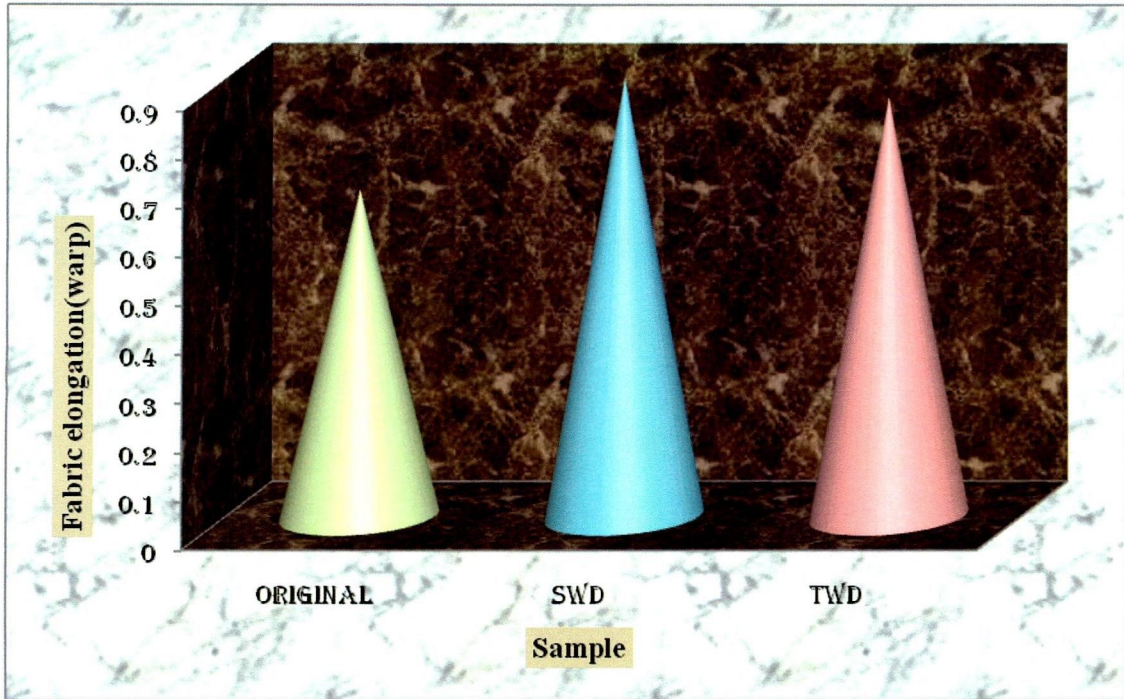


FIGURE XII

FABRIC ELONGATION (WARP)

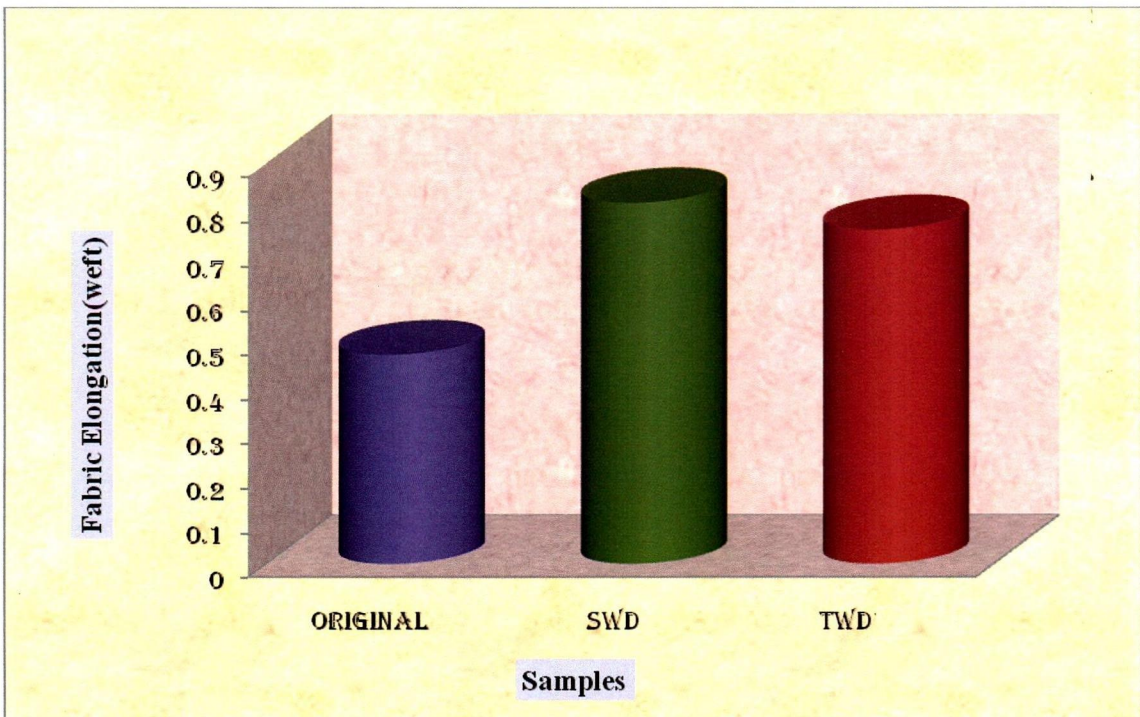


FIGURE XIII

FABRIC ELONGATION (WEFT)

From the Table XIX and Figure XII, it is clear that there is an increase in elongation of both the dyed samples. The increase in elongation of sample SWD and TWD are 34.3%, and 28.3% along warp direction, when compared over original.

Statistical analysis shows that there is a significant difference at 1% level among the samples.

Fabric Elongation (weft)

Elongation of the samples O, SWD, and TWD in weft direction are presented in Table XX and Figure XIII.

TABLE XX

FABRIC ELONGATION (WEFT)

S.No	Samples	Mean wt in (GSM)	Loss / gain over original	% loss / gain over original
1.	Original	0.47	-	-
2.	SWD	0.81	0.34	72.3
3.	TWD	0.75	0.28	59.5

Anova for Fabric Elongation (Weft) % Loss or Gain

	Sum of Squares	Degree of freedom	Mean Square	F
Between Groups	0.333480	2	0.166740	1191.0000**
Within Groups	0.335440	14	0.023960	
Total	0.668920	16	0.190700	

**Significant at 1% level

From the Table XX and Figure XIII, it is clear that there is an increase in elongation of all the dyed samples. The increase in elongation of sample SWD and TWD are 72%, and 60% respectively along weft direction, when compared over original.

Statistical analysis shows that there is a significant difference at 1% level among the samples. Thus it could be concluded that treated water can be used for dyeing effectively.

4.5.2.6. Abrasion resistance

Abrasion resistance and analysis of variance of the samples O, SWD and TWD are presented in Table XXI and Figure XIV.

TABLE XXI

ABRASION RESISTANCE

S.No	Samples	Mean wt in (GSM)	Loss / gain over original	% loss / gain over original
1.	Original	0.003	-	-
2.	SWD	0.002	-0.001	3.3
3.	TWD	0.004	0.001	3.3

Anova for Abrasion Resistance % Loss or Gain

	Sum of Squares	Degree of freedom	Mean Square	F
Between Groups	0.000011	2	0.000005	10.1935**
Within Groups	0.000018	14	0.000001	
Total	0.000029	16	0.000006	

**Significant at 1% level

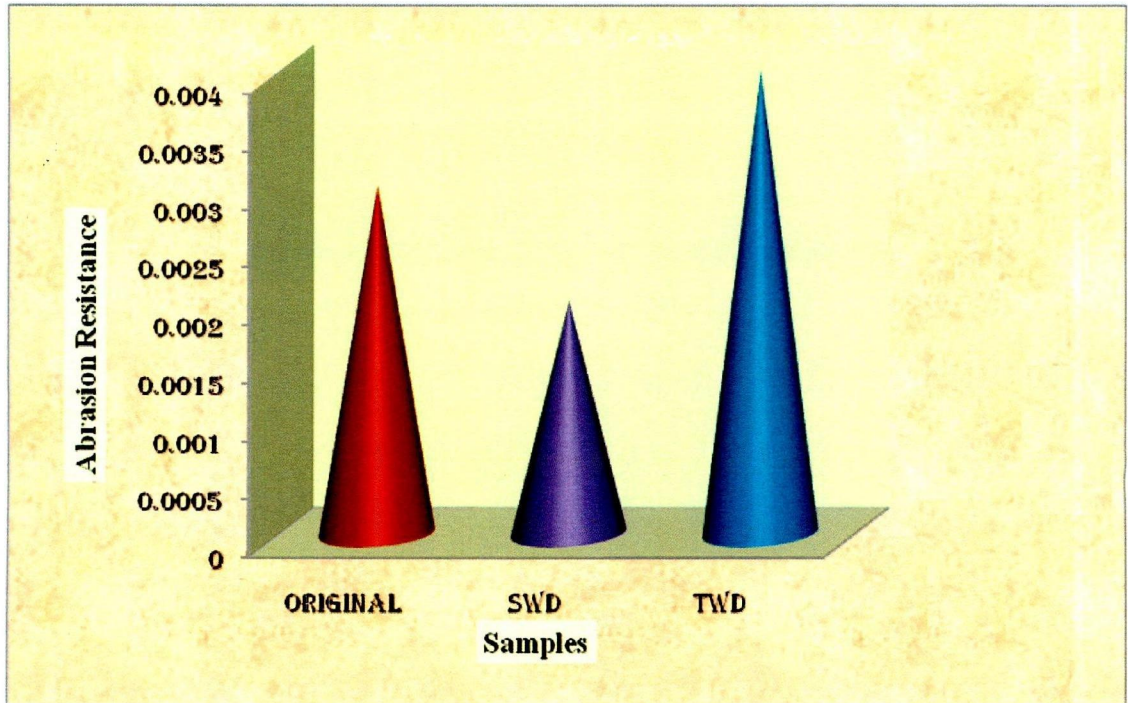


FIGURE XIV
ABRASION RESISTANCE

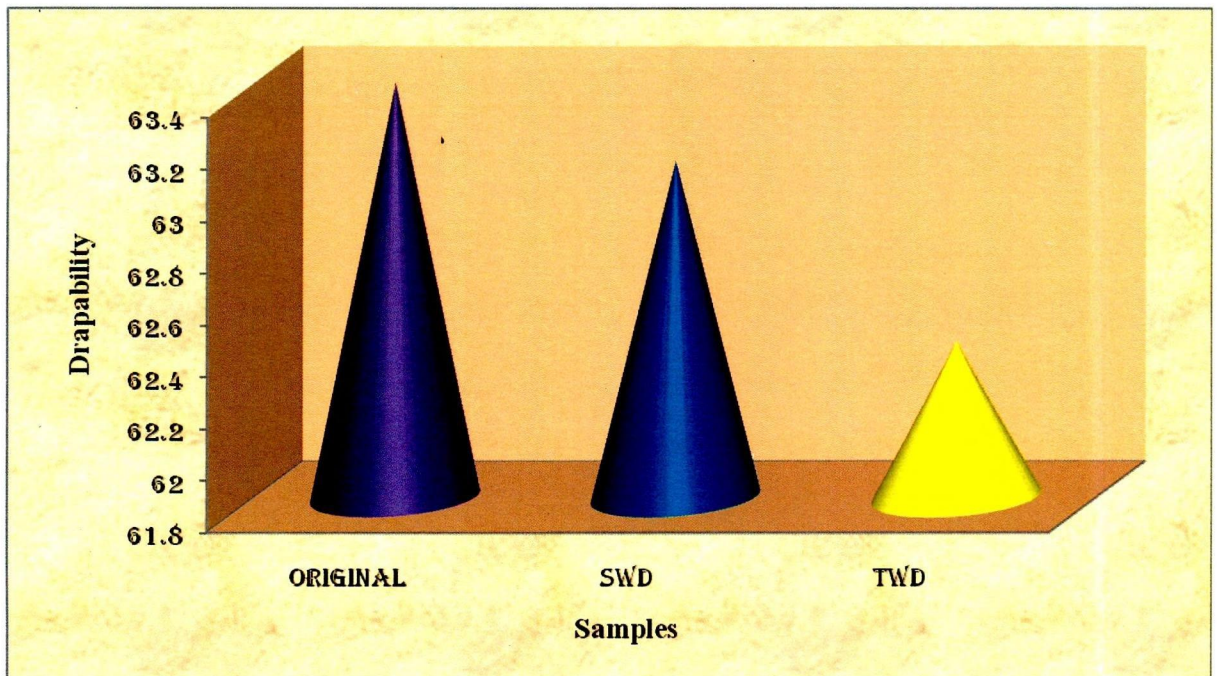


FIGURE XV
DRAPABILITY

From Table XXI and Figure XIV it is clear that the abrasion resistance of the samples SWD, and TWD was better when compared to the original. Sample SWD and TWD showed the same abrasion resistance as 3% .Hence it can be concluded that the water did not affect the samples.

Statistical analysis shows that there is a significant difference at 1% level among the samples.

4.5.2.7. Drapability

Results of drape co-efficient of the samples O, SWD, and TWD are presented in Table XXII and Figure XV.

**TABLE XXII
DRAPABILITY**

S.No	Samples	Mean wt in (GSM)	Loss / gain over original	% loss / gain over original
1.	Original	63.4	-	-
2.	SWD	63.1	-0.3	0.47
3.	TWD	62.4	-1	1.5

Anova for Drapability % Loss or Gain

	Sum of Squares	Degree of freedom	Mean Square	F
Between Groups	2.777333	2	1.388667	95.7701**
Within Groups	2.937333	14	0.209810	
Total	5.714666	16	1.598477	

**Significant at 1% level

From Table XXII and Figure XV it is clear that the original and dyed samples had more or less similar drape co-efficient except TWD. The drape co-efficient of sample TWD is lowered by 1.5% over original.

Statistical analysis shows that there is a significant difference at 1% level among the samples.

4.6.2.8. Absorbency test

The results related to absorbency of the samples are presented in Table XXIII and Figure XVI.

From Table XXIII, the capillary rise was maximum in SWD with rise of 2.5 cm per minute followed by TWD of 2.1 cm per minute.

TABLE XXIII
ABSORBENCY TEST

S.No	Samples	Mean capillary rise cm/ 60 secs	Drop test (sec)	Sinking test (sec)
1.	Original	4.3	6	4
2.	SWD	2.5	22	20
3.	TWD	2.1	10	27

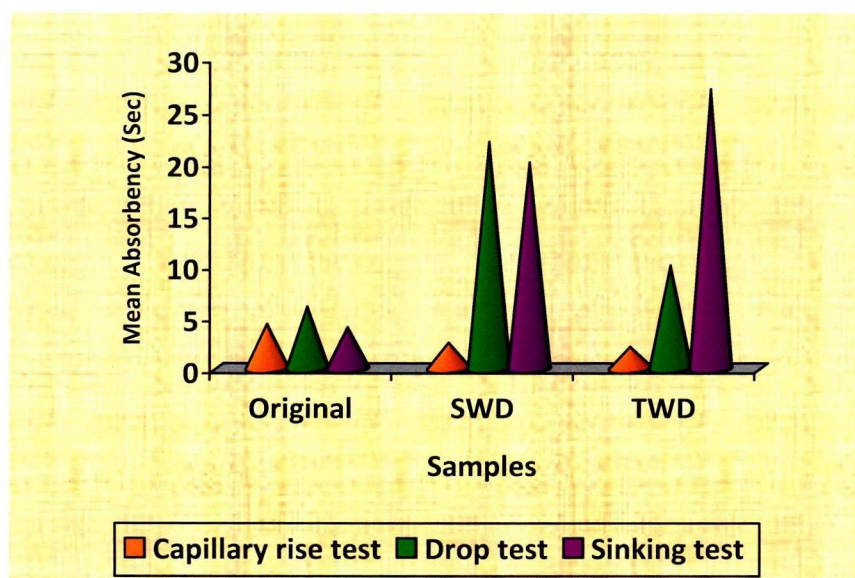


FIGURE XVI
ABSORBENCY TEST

In drop test, SWD took 22 seconds to absorb a drop of water followed by TWD (10 seconds), with regard to sinking test, SWD sample took 20 seconds to sink and TWD sample took 27 seconds respectively.

4.5.2.9. Color fastness tests

Color fastness results of dyed fabric to sunlight, pressing, crocking and washing are presented in Table XXIV.

TABLE XXIV

COLOR FASTNESS TEST

S. No.	Samples	Sunlight	Pressing		Crocking		Washing	
			Dry staining	Wet staining	Dry staining	Wet staining	Color change	Staining
1.	SWD	5	5	4	5	4	5	5
2.	TWD	5	5	4	5	4	5	5

From Table XXIV it is evident that all the dyed samples SWD and TWD have good fastness to sunlight. With regard to pressing all the samples had excellent fastness to pressing in dry condition and staining. Hence it could be concluded that the samples SWD and TWD had excellent fastness to sunlight, pressing, crocking and washing.