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EFFECT OF ETHYLENE ON ANABAENA DOLIOLUM

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ABSTRACT

Study the effect of Citrate –phosphate buffer as well as the duration of treatment of the buffer on the growth and development of A. doliolum. The alga was treated with Citrate-phosphate buffer for ½ hrs, 1.30 hrs, 3 hrs,6 hrs 12 hrs and 24 hrs.

Different concentrations of ethylene was prepared, 12 tubes were prepared for culture of alga for treatment with 100µg/ml,10µg/ml,1µg/ml,0.1µg/ml,0.01µg/ml,0.001µg/ml of ethylene. Each culture had two sets, one was control culture and the other was the treatment culture. The treatment was allowed for 6 hours in buffer solution containing different concentrations of ethylene. After six hours of C_2H_4 treatment, the alga from each treatment was centrifuged, washed and cultured in BG-11 for studying growth and differentiation in A. doliolum treated with different concentration of C_2H_4.

INTRODUCTION

The discovery of ethylene as a plant growth regulator might be said to have occurred as the inverse of the discoveries leading to the other plant hormones. Since very early days it was considered to be an exogenous chemical with strong physiological effects, and only gradually over a series of decades did it become evident that this compound is in fact a natural plant hormone (Leopold and Kriedermann, 1975). The story began when the observation of Neljubow (1901) that ethylene gas can markedly alter the tropistic responses of roots. The synthetic gas was observed by Rosa (1925) to be fairly effective in breaking potato dormancy, by Denny (1924) to be highly effective is inducing fruit ripening by Zimmerman et.al .(1931) in inducing leaf abscission ,and by Rodrigues(1932) in inducing flowering in pineapple plants. These empirical experiment suddenly took on a different quality when Gane (1934) established that ethylene is actually a natural product of ripening fruits opened that its presence might therefore count for their stimulatory mutual ripening effect. Denny and Miller (1935) found evidence of ethylene production not only by ripening fruits but also by flowers, seeds leaves and even roots. It was also found to be a natural product in self-branching celery (Nelson and Harvey, 1935).

The natural extrapolation from these facts that ethylene exerts proformed regulatory activity and that ethylene is formed naturally in plants in amounts sufficient to bring about regulatory effects was that ethylene might be included in the domain of plant hormone Crocker et al (1935) proposed the idea that ethylene might be considered as a plant hormone. Went and Thimann (1937) strongly objected to the hormonal nature of ethylene.

RESEARCH METHODOLOGY

EXPERIMENT-1

The solution of ethylene was prepared by dissolving Etherel (2-chloroethylsulphonic acid) in citrate phosphate buffer (pH3.8). Since the hormone has been prepared in Citrate-phosphate buffer (Ph3.8), the study of effect of ethylene on A. doliolum was undertaken in two phases:

1) Experiment to study the effect of Citrate –phosphate buffer as well as the duration of treatment of the buffer on the growth and development of A. doliolum. The alga was treated (cultured) with Citrate-phosphate buffer for 1/2hr, 1.30 hrs, 3 hrs, 6 hrs, 12 hrs and 24 hrs (Figs. 4.49-4.50).

Preparation of citrate-phosphate buffer (pH. 3.8):
Stock solution;
A:- 0.1M solution of citric acid (19.21g/1000ml)
B:- 0.2M solution of Na_2HPO_4 (53.658 OF $Na_2HPO_47H_2O$/Liter or 71.7g of $Na_2HPO_412H_2O$/liter)
19.21 g…….1000 ml
1.92 g…….100 ml
Citric 100 ml

$Na_2HPO_47H_2O$-2.68gm/50ml

To prepare 100 ml Citrate-Phosphate buffer:-

To prepare 100 ml Citrate –Phosphate buffer, 1.92 g citric acid in 100 ml distilled water and 2.68 gm Na_2HPO_4 in 50 ml distilled water. Added 32.3 ml of Citric acid and 17.7 ml solution of Na_2HPO_4, mixed well and the solution was made 100 ml by adding distilled water. The Citrate –Phosphate buffer solution had pH value3.8.

RESULT:-

The study of effect of Citrate-Phosphate buffer on the alga was important because it had a pH value of 3.8 and in general the alga grows in BG-11 medium at pH 7.2 Fig 4.51 shows the growth pattern of A. doliolum cultured in Citrate-Phosphate buffer for different periods of time i.e. 30 min, 90 min, 3 hrs, 6 hrs, 12 hrs & 24 hrs. It is evident from the Fig. 4.51, the alga shows the best growth performance when treated with Citrate –Phosphate buffer for 6 hrs. Fig.4.52 shows the absorption spectrum of acetone extracts of Citrate-Phosphate buffer treated A.doliolum for different periods of time.

EXPERIMENT: 2-

Now different concentrations of ethylene was prepared as follows:

METHOD:

Stock of ethylene = 4,80,000 µg/ml

A. 1 ml from stock (Bottle) + 9ml buffer - 48,000 µg/ml
B. 1 ml of A + 9 ml buffer - 4,800 µg/ml
C. 1 ml of B + 9 ml buffer - 480 µg/ml

$S_1V_1 = S_2V_2$

100 µg/ml 480 x V_1 = 100 x 10

480 x V_1 = 1000

Or, $V_1 = 1000/480$

$V_1 = 100/48$

= 2.08 ml + 7.92 ml buffer

= (100 µg/ml)

D. 2ml of C + 8ml buffer = 10 ml = 100 µg/ml …… (1)
E. 1ml of D + 9ml buffer = 10 ml = 10 µg/ml …….. (2)
G. 1ml of F + 9 ml buffer = 10 ml = 0.1 µg/ml ……. (3)
H. 1ml of G + 9ml buffer = 10 ml = 0.01 µg/ml …….(4)
I. 1ml of H + 9ml buffer = 10 ml = 0.001 µg/ml……..…..(5)

12 tubes were prepared for culture of alga for treatment with 100 µg/ml, 10 µg/ml, 1 µg/ml, 0.1 µg/ml, 0.01 µg/ml, 0.001 µg/ml of ethylene. Each culture had two sets, one was control culture and the other was the treatment culture. The treatment was allowed for 6 hours in buffer solution containing different concentration of ethylene. After six hours of C_2H_4 treatment, the alga from each treatment was centrifuged, washed and cultured in BG-11 for studying growth and differentiation in A. doliolum treated with different concentration of C_2H_4.

RESULTS AND DISCUSSION

The control culture of A.doliolum showed a heterocyst frequency of 8.84% however the alga treated with 100µg/ml C_2H_4 and 10µg /ml showed the average frequency of heterocyst higher than control filaments. The filaments of A.doliolum in the presence of 100µg /ml showed fragmented filaments with heterocysts (Fig 4.54 and Fig 4.55). In terms of pigment contents Fig4.53 the control shows better performance than the culture treated with 100µg/ml C_2H_4. However, the increase in the heterocyst frequency in the presence of C_2H_4 (100µg/ml) was observed. The frequency of heterocyst was 11.38% as compared to 8.84% of the control filament. The heterocyst showed prominent polar nodules. Some free heterocysts and short filaments have also been observed (Fig 4.57 a-c). The short, fragmented filaments were found in clusters. Likewise, the alga showed increase in heterocyst frequency as compared to control and was observed to be 10.2% in the presence of 10µg/ml C_2H_4. A new tendency was observed in the alga in the presence of 10µ/ml C_2H_4. i.e. a tendency towards clumping and contorted morphology (Fig.4.57). The filaments were long, heterocystous with prominent polar nodules, and showing highly contorted morphology. In the presence of lower concentrations of C_2H_4 the alga had a lower frequency of heterocysts i.e. 8.3%, 8.27%, 6.74% and 6.12% in the presence of 1 µg/ml, 0.1µg/ml 0.01 µg/ml and 0.001µg/ ml C_2H_4.

Table-1

Effect of Citrate-phosphate buffer (pH 3.8) on A. doliolum

	½ hr.	1½ hr.	3 hr.	6 hr.	12 hr.	24 hr.
Control	0.01	0.03	0.01	0.01	0.02	0.03
Culture	0.03	0.05	0.04	0.06	0.08	0.07
Control	0.01	0.03	0.04	0.04	0.03	0.04
Culture	0.07	0.08	0.08	0.16	0.15	0.1
Control	0.04	0.04	0.05	0.04	0.04	0.04
Culture	0.1	0.2	0.14	0.25	0.18	0.13
Control	0.07	0.04	0.05	0.04	0.05	0.04
Culture	0.16	0.21	0.18	0.26	0.02	0.15

Table-2

C_2H_4 Absorption spectrum of pigments extracted from Citrate-Phosphate buffer (pH 3.8) treated A.doliolum

		400	420	440	460	480	500	520	540	560	580	600	620	640	660	680	700
½ hr.	Control	0.06	0.18	0.4	0.38	0.46	0.34	0.24	10.12	0.08	0.08	0.08	0.1	0.09	0.18	0.3	0.05
	Treatment culture	0.12	0.32	0.49	0.49	0.22	0.31	0.19	0.05	0.04	0.06	0.07	0.1	0.09	0.18	0.27	0.04
1½ hr.	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Treatment Culture	0.19	0.45	0.9	0.00	0.42	0.66	0.41	0.18	0.09	0.13	0.17	0.23	0.21	0.38	0.72	0.7
3 hr.	Control	0.07	0.11	0.2	0.21	0.27	0.9	0.42	0.13	0.06	0.11	0.14	0.22	0.19	0.33	0.7	0.08
	Treatment Culture	0.1	0.22	0.49	0.00	0.10	0.04	0.03	0.02	0.09	0.13	0.11	0.15	0.13	0.26	0.4	0.08
6 hr.	Control	0.00	0.00	0.00	0.00	0.04	0.04	0.03	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.00
	Treatment Culture	0.1	0.22	0.54	0.55	0.44	0.3	0.2	0.12	0.06	0.07	0.08	0.12	0.11	0.21	0.33	0.05
12 hr.	Control	0.01	0.02	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.01	0.02	0.00
	Treatment Culture	0.12	0.28	0.7	0.39	0.79	0.5	0.55	0.21	0.1	0.13	0.12	0.17	0.15	0.28	0.45	0.06
24 hr.	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Treatment culture	0.08	0.15	0.15	0.53	0.7	0.6	0.38	0.16	0.09	0.11	0.1	0.15	0.14	0.23	0.59	0.07

Table-3

Control

SL. No.	No. of Cells	No. of heterocyst	% of heterocyst
1	35	2	5.7
2	5	1	20
3	30	2	6.6
4	28	2	7.1
5	32	4	12.5
6	12	1	8.3
7	28	1	3.2
8	91	6	6.5
9	13	2	15.3
10	61	2	3.2

Average-8.84%

Table-4

Effect of C_2H_4 on A.doliolum (Concentration-100µm)

SL. No.	No. of cells	No. of heterocyst	% of heterocyst
1	14	1	7.1
2	54	3	5.5
3	73	5	6.9
4	5	2	40
5	29	2	6.8
6	98	5	5.1
7	49	3	6.1
8	51	6	11.7
9	12	1	16.6
10	25	2	8

Average-11.38%

Table-5

Concentration-(10µg)

SL. No.	No. of Cells	No. of heterocyst	% of heterocyst
1	36	3	8.3
2	6	1	16.6
3	15	1	6.6
4	19	1	5.8
5	22	2	9.09
6	57	3	5.2
7	42	2	4.7
8	18	3	16.6
9	10	2	20
10	30	3	10

Average- 10.2 %

Table-6

Concentration- (1µg)

SL. No.	No. of Cells	No. of heterocyst	% of heterocyst
1	17	2	11.7
2	15	2	13.3
3	17	1	5.8
4	44	3	6.8
5	15	2	13.3
6	46	2	4.3
7	32	2	6.2
8	12	1	8.3
9	42	2	4.7
10	22	2	9.09

Average-8.3%

Table-7

Concentration-(0.1µg)

SI. No.	No. of Cells	No. of heterocyst	% of heterocyst
1	28	2	7.1
2	15.2	7	4.6
3	90	5	5.5
4	29	1	3.4
5	8	1	12.5
6	29	3	10.3
7	71	6	8.4
8	46	5	10.8
9	16	2	12.5
10	13	1	7.6

Average-8.27%

Table-8

Concentration-(0.01µg)

SL. No.	No. of Cells	No. of heterocyst	% of heterocyst
1	49	5	1.2
2	5	1	20
3	83	4	4.8
4	19	1	5.2
5	13	1	7.6
6	139	8	5.7
7	23	1	4.3
8	16	1	6.2
9	64	3	4.6
10	38	3	7.8

Average-6.74%

Table-9

Concentration-(0.001µg)

SL. No.	No. of Cells	No. of heterocyst	% of heterocyst
1	30	1	3.3
2	25	2	8
3	19	1	5.2
4	60	2	3.3
5	12	1	8.3
6	23	1	4.3
7	125	6	4.8
8	17	1	5.8
9	60	1	1.6
10	6	1	16.6

Average-6.12%

CONCLUSION

It was observed that production of ethylene was significant when the fungus was cultured under static conditions (without shaking) and it might have been related to the surface development of a mycelial mat. Later, it was also found that P. digitatum is capable of producing small or large quantities of ethylene from different substrates depending on the type of medium and condition of culture under which the fungus was grown. Thomas and spencer showed that shake cultures of yeast, saccharomyces serevisiae were induced by L-methionine to produce ethylene ,and in addition ,as with P. digitatum in shake cultures, L-methionine was also a substrate for ethylene in this system . The production of methionine-induced ethylene was increased by glucose in the medium.
Ethylene production from methionine has been reported in Escherichia coli from Mucor hiemalis and other microbes isolated from soils. There are some doubts about the physiological or metabolic nature of ethylene production by these micro-organisms especially since they require light and are generally stimulated by reduced iron conditions which convert methionine to ethylene non-enzymatically.

Currently there has been considerable research on the production, metabolism and function of Ethylene in soils and an oxygen-ethylene cycle was proposed which may have a bearing on the biological balance in soils. It is interesting to note that production of ethylene in soils is associated with anaerobiosis and yet it is a process that requires oxygen in higher plant. Russo et. al showed that ethylene mediates the avoidance response of the sporangiophore of the fungus Phycomyces blakesleeanus. Ehylene induces the tendency for clumping, ethylene also increased the frequency of heterocyst.

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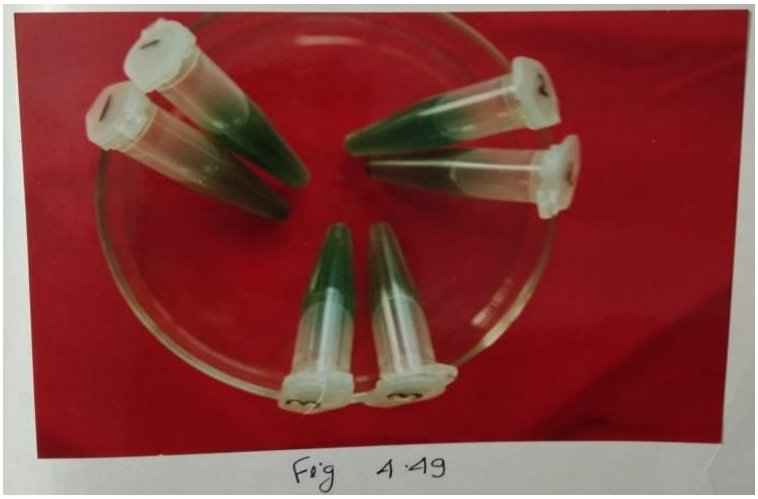
FIGURES:-



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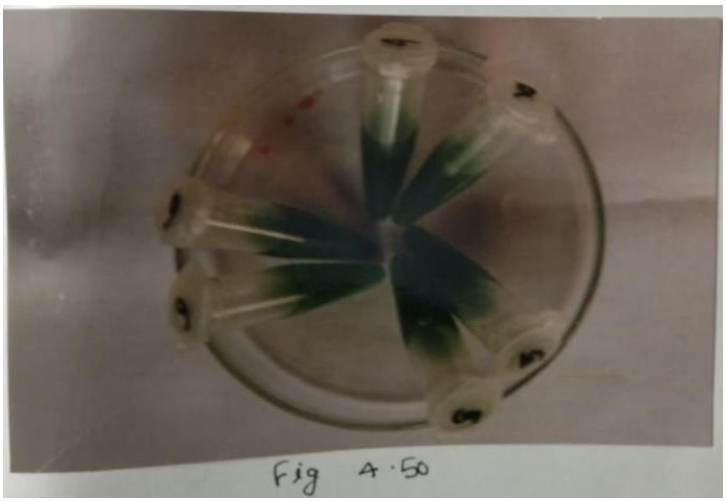

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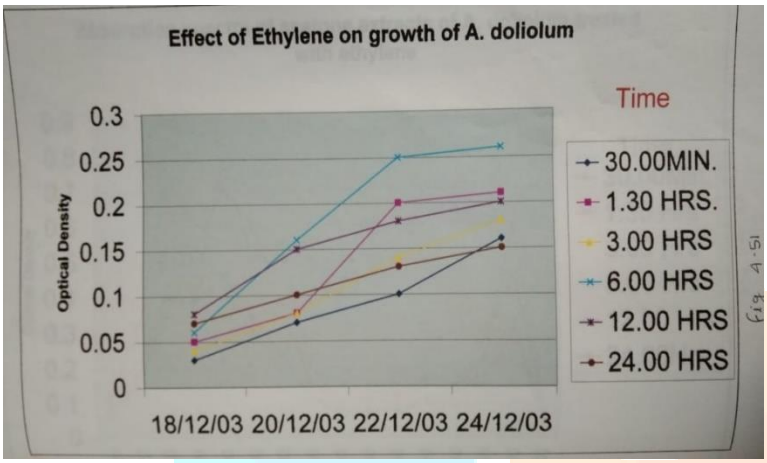

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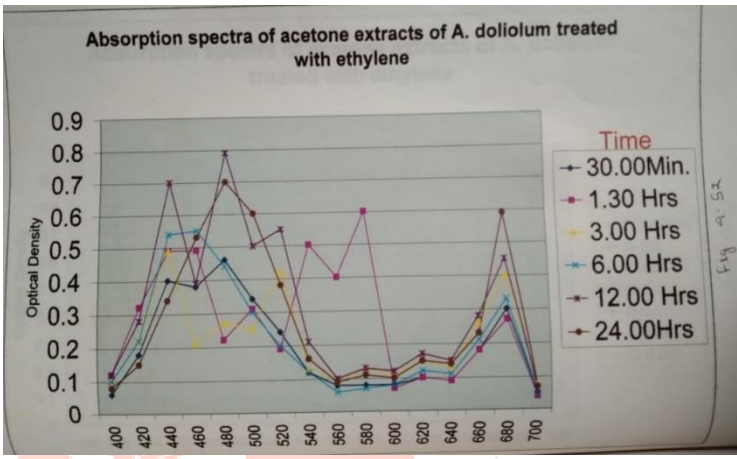
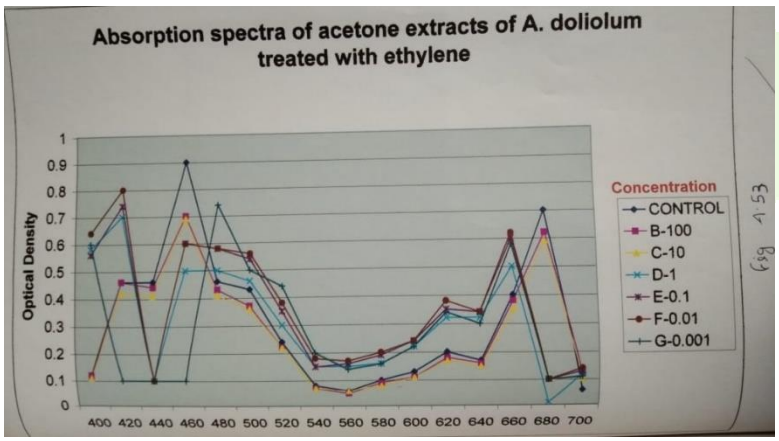


Fig 4.52



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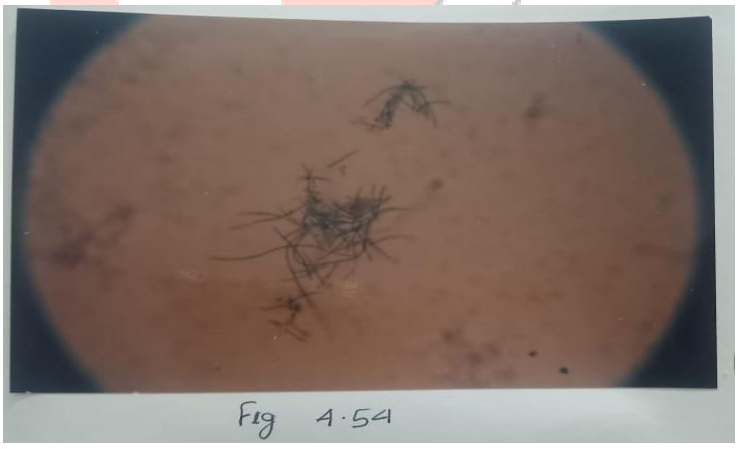

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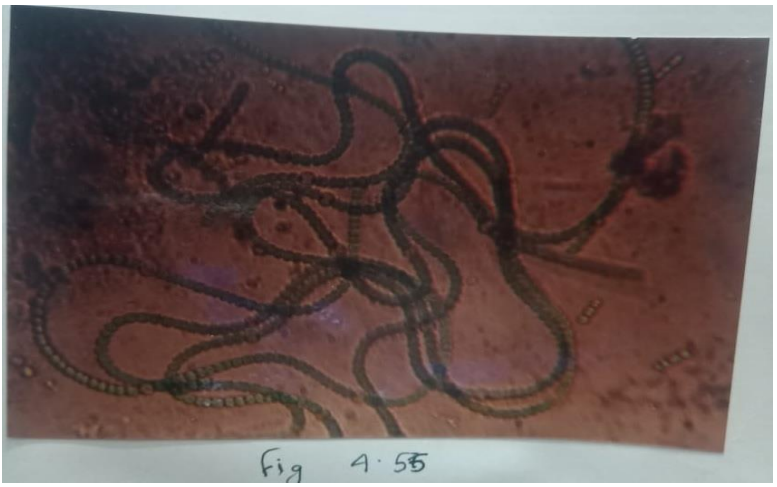

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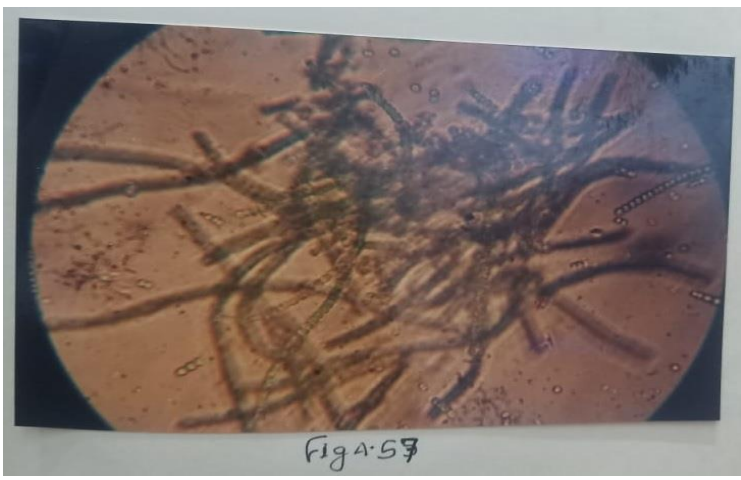

Fig 4.56