# DISINFECTION OF WATER BY USING NATURAL DISINFECTANTS (ACACIA NILOTICA)

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#### **ABSTRACT:**

Water is the most important resource and is vital for all life on the earth. The well-being and development of our society is dependent on the availability of water. The most precious resource is sometimes scarce, sometime abundant and is always very unevenly distributed. In the ancient time human required water for the drinking, bathing, cooking etc. but with the advancement in the civilization, the utility of water enormously increased. Water is the good carrier of disease germ, and may be responsible for water borne diseases. Therefore, water which is required by public should be wholesome and must be free from pathogens. The water borne disease falls into five categories. According to the nature of organisms, causing disease viz. Bacteria, protozoa, worms, viruses and fungi. The water borne diseases are typhoid fever, paratyphoid fever, bacillary dysentery and cholera. Therefore it is very important that water works must remove the bacteria's from the water and make it wholesome. This particular research work is carried out for the disinfection of water by using natural plants or plant herbs. By using natural plant or plant herbs we can kill the pathogenic bacteria from the water and make it safe for the user. These natural Disinfectants are easily available and economical.

In rural area, the peoples are using the water from the rivers or wells, in such area the people can easily used such natural plants & can prevent the water borne diseases. These treatments are also called as low cost treatment methods. As compare to other methods of disinfection, disinfection of water by using natural plant are very cheap method & also can give the employment facility to the people.

#### **1.RESEARCH WORK**

This Particular research work is carried out in Neeri Research Institute Nagpur, for the disinfection of water by using natural plants or plant herbs. By using natural plant or plant herbs we can kill the pathogenic bacteria from the water and make it safe for the user. These natural Disinfectants are easily available and economical.

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- 2. AIM OF THE WORK:
- 1) To kill the pathogenic bacteria by using natural disinfectant e.g. Tamarindus Indica. Acacia Nilotica.
- 2) To prevent the waterborne disease by using natural disinfectants.
- 3) To protect the human life from contaminated water.
- To reduce the cost of treatment by using such natural disinfectants.
- 5) To provide employment facility to the millions of people.
- 6) The disinfection of water by using natural disinfectant is the low cost treatment.
- 7) To achieve economy.
- 8) Ecofriendly in nature.
- 9) In rural area, these plants are easily available.

**10**) In rural area, the peoples are using the water from well or any other sources of water without any treatment, if these peoples knows these plants are used for the disinfection of water, then they can used these plants for the disinfection of water.

#### **3 METHOD:**

#### **3.1 SAMPLE COLLECTION**

Sample site was selected from the Neeri Nala sewage water. This nala is situated in the campus of Neeri research institute, Nagpur. Sample was collected in a clean sterilized stopper bottle. Nala water sample was taken the bottle under a septic condition and sample butte was transported laboratory immediately & refrigerated.

#### **3.2 MEMBRANE FILTER TECHNIQUE FOR COLIFORM:**

The method consists of filtering the sample of water through a filter membrane of uniform microscopic pores small enough to retain all the coliforms and other bacteria. The filtration procedure consists of passing the measured quantity of water sample through the membrane under partial vacuum. After the sample has been filtered, the funnel is rinsed three times with 20-30 ml volume of sterile buffered dilution water. The membrane is then lifted up with sterile foreceps, gently

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rolled, grid side up, on to the surface of an absorbent pad containing M-endo medium. The membrane cultures are incubated at 37°C for 20 hours, in an inverted position in the incubator with 100 % humidity. The colony counting exhibit a metallic-appearing surface luster. The colony counting is done either with the help a low power microscope or with the help of any suitable colony counter. The estimated Coliform density of the sample is recorded in terms of Coliform per 100 ml sample, using relaxation:

### 3.3 NATURAL DISINFECTANT-ACACIA NILOTICA

Acacia nilotica- family- Mimosaceae (Babul) : The distribution of acacia nilotica is throughout the India in deciduous forests, also cultivated. A moderate sized tree upto 10 m in height with dark brown or black Longitudinally fissured rough bark and reddish brown heartwood.

Parts used: The bark of acacia nilotica is useful in kapha and pitta, ulcer, chronic dysentery, diarrhea, leprosy, leucoderma, skin diseases, burning sensation, cough, Bronchitis.

The gum of acacia nilotica is sweet and it is used a liver tonic is also useful in vata and pitta, cough, asthma, diarrhea, dysentery, leprosy, burns, intermittent, fevers and general debility. The acacia nilotica is a medicinal plant from the reference of ayurveda.



# 4.LABORATORY WORK FOR LEAVES OF ACACIA NILOTICA (JUICE FORM)

- 1) Take the green leaves of acacia nilotica.
- 2) Then green leaves are grinded in to the grinding machine. After grinding process the green leaves are become converted into the juice form.
- 3) Prepare the 30 plates of M-Endo,each plate having 20 ml capacity e.g. for 980 ml distilled water dissolve 51 gms of M-Endo media and add 20 ml alcohol for 100 ml distilled water. Therefore for 30 plated means for 600 ml distilled water, calculate the quantity of M-Endo media and also calculate the quantity of alcohol for 600 ml capacity.
- 4) Again prepare the 30 plated of M-FC media, each plate having 20 ml capacity e.g. for 1000 ml distilled water dissolve 52.1 gms of M-FC media. Therefore for 30 plated means for 600 ml distilled water, calculate the quantity of M-FC media.

- 5) Pour the M-Endo media in 30 petridishes for Total coliform and pour the M-FC media in 30 petridishes for faecal coliforms & keep it for sometimes to solidify them.
- 6) Mark the various hours of natural disinfectant on petridish
- 7) Take 2 conical flasks for control and sample.
- 8) Take 99 ml distilled water in each 1 conical flask for control and also take 99 ml distilled water in another flask for sample
- 9) Mark also on the conical flask for e.g, C:99 ml distilled water+ 1 ml sewage and S: 99 ml distilled+ 1 ml sewage+ 1 ml juice of acasia nilotica (juice form of leaves of acacia nilotica).
- 10) Then add 1 ml quantity of juice of acacia nilotica(juice form of leaves of acacia nilotica)in each respective conical flask for sample not for control.
- 11) Wrap the 60 petriplates with brown paper and tie it properly tipbox,membrane filter assemble and conical flask containing 99 ml distilled water for control and 99 ml distilled water + 1 ml juice of acacia nilotica (juice form of leaves of acacia nilotica ) are well sterilized in autoclave at 121°c at 15 lbs pressure for 15 minutes.
- 12) Then add 1 ml of sewage in 99 ml distilled water in 1 conical flask for control and also add 1 ml of sewage in 99 ml distilled water in 1 conical flask for sample also.
- 13) Note the time immediately after the addition of sewage in sample and control.
- 14) Place the filter paper with the help of sterile forceps on the sterile membrane.
- 15) After 0 hours, from the time at which the sewage is added in control and sample, take 1 ml form the sample(S:1ml+ 99 ml distilled water+1ml sewage) with the help of micropipette with tip and passing the sample through the filter under vaccum.The filter should be rinsed by filtration. Two or three times with 20 ml or 30 ml of sterile water.
- 16) Unlock the assemble and remove the funnel and remove the filter paper by sterile forceps and place it on the sterile pad or agar petridish i.e. S: 0 hr of M-Endo media with a rolling motion to avoid the entrapment of air.
- 17) Again place the filter paper with the help of sterile forcep on the sterile membrane.
- 18) Then take the 5 ml from the sample (99 ml distilled water+ 1ml of juice of acacia nilotica (juice form of leaves of acacia nilotica + 1 ml sewage) with the help of micropipette with same tip and passing the sample through the filter under vaccum. The filter should be rinsed by filtration two. Or three times with 20 ml to 30 ml of sterile water.

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- 19) Unlock the assembly and remove the funnel and remove the filter paper by sterile forceps and place it on the sterile pad or agar Petridish i.e. S: 0 hr of M-FC media with rolling motion to void the entrapment of air.
- 20) Remove the tip from the micropipette and take another tip and fix to the micropipette. (When change from sample to control)
- 21) Wash the whole assembly of membrane filter with sterile water.
- 22) Again place the filter paper with the help of sterile forceps on the sterile membrane.
- 23) Again take 1 ml from the control (99 ml distilled water+ 1ml sewage) with the help of micropipette with the new tip and passing the control through the filter under vacuum. The filter should be rinsed by filtration, two or three times with 20 ml to 30 ml of sterile water.
- 24) Unlock the assembly and remove the funnel and remove the filter paper by sterile forceps and place it on the sterile pad or petridish or M-Endo agar media i.e. C:0 hr with rolling motion to avoid the entrapment of air. Wash again with sterile water.
- 25) Again place the filter paper with the help of sterile forceps on the sterile membrane.
- 26) Again take the 5 ml form the control (99 ml distilled water + 1ml sewage ) with the help of micropipette with the same tip which is used for M-Endo media and passing through the filter under vacuum. The filter should be rinsed by filtration, two or three with 20 ml to 30 ml sterile water.
- 27) Unlock the assembly and remove the funnel and remove the filter paper with sterile pad or Petridis of M-FC media i.e. C:0 hr with rolling motion to avoid the entrapment of air.
- 28) Repeat the above procedure after 0 hr,15 min, 30min,1hr,
  2hr,3hr,4hr,5hr,6hr,7hr,8hr,9hr,10hr,11hr.12 hr for

both the sample and control.

- 29) All the petriplates of M-Endo media(S, C) are kept in incubator at 28°c for 24 hours.
- 30) All the petriplates of M-FC media are kept in incubator at  $44^{\circ}$ c for 24 hours.
- 31) After 24 hours, the colonies are formed on the filter paper on which the bacteria are entrapped. The green metallic sheen and are pink to rose red in color is the total coli forms and the colonies which are blue in color is the faecal coli forms.
- 32) Count the colonies manually or with the help of digital colony counter.
- 33) The estimated coli form density of the sample is recorded in term of coli forms per 100 ml sample using the relation.

coliform colonies per 100 ml =  $\frac{\text{coliform colonies counted} \times 100}{\text{ml sample filtered}}$ 



# 5. TOXICITY TEST:

Toxicity test is generally carried out to check the toxic effect of this natural disinfection on the aquatic life. These tests are carried out in laboratory on the fish e.g. Guppy fish.

# **5.1 LABORATORY WORK: TOXICITY TEST FOR JUICE OF LEAVES OF ACACIA NILOTICA (JUICE FORM):**

- The following procedure adopted for the toxicity test
- 1) Take the 1 tank of at least 5 liters capacity
- Fill up these tanks up to 3 liters with dechlorinated water.
- If the water containing chlorine then dechlrinates the water by keeping the water open to the atmosphere for one day.
- 4) The 4 fish are kept in each tank for at least 7 days to suit the environment.
- 5) Provide the continuous aeration with the help of aerators
- 6) Then add the 30 ml juice of leaves of acacia nilotica in the tank.
- 7) Note down the time at which the dose is added in the tank.
- 8) Check the toxic effect for 96 hours from the time of adding the dose in the tank.
- 9) If the fish are alive then the disinfectant is not toxic to the aquatic life.
- 10) If the fish will die after the addition of dose of juice of leaves of leaves of acacaio nilotica( juice form of acacia nilotica) then the leaves of acacia nilotica is toxic to the aquatic life



#### 6. OBSERVATIONS:

3

30 min

# TABLE 1 AND 2 : OPTIMUM PERIOD OF JUICE OFLEAVES OF ACACIA NILOTICA (JUICE FORM)

Sample taken  $\rightarrow$  Juice of leaves of acacia nilotica (juice form)  $\rightarrow$  1 ml

Media used  $\rightarrow$  M-Endo  $\rightarrow$  Total coliforms.

M-FC $\rightarrow$  Faecal coliforms or thermotolerent coliform Reading after 24 hours

 $\text{M-Endo} \rightarrow 1\text{ml sample} \rightarrow [\text{S, C}] \rightarrow 28^{0}\text{-}30^{0}\text{c}$ 

M-FC $\rightarrow$ 5ml sample $\rightarrow$ [S, C] $\rightarrow$ 44°c							
		SAMPL	M – END	M - FC			
	SR.NO	Ε	1 ML	CFU/100	5 ML		
		TIME	TML	ML	3 ML		
	1	0 hr	13	1300	08		
	2	15 min	08	800	04		

03

B.T	CONTRO				Contra La che	
SR	CONTRO	M – ENDO		M - FC		
15	12 hou	r 00	00	00	-00	
14	11 hou	r 00	00	00	00	
13	10 hou	r 00	00	00	00	
12	9 hour	00	00	00	00	
11	8 hour	00	00	00	00	
10	7 hour	00	00	00	00	
9	6 hour	00	00	00	00	
8	5 hour	00	00	00	00	
7	4 hour	00	00	00	00	
6	3 hour	00	00	00	00	
5	2 hour	00	00	00	00	
4	1 hour	00	00	00	00	

300

SR	CONTRO	M – ENDO		M - FC	
.N O	L TIME	1 ML	CFU/100 ML	5 ML	CFU/100 ML
1	0 hr	15	1500	10	200
2 .	15 min	18	1800	12	240
3	30 min	20	2000	15	300
4	1 hour	22	2200	18	360
5	2 hour	27	2700	21	420
6	3 hour	30	3000	23	460
7	4 hour	33	3300	25	500
8	5 hour	35	3500	28	560
9	6 hour	38	3800	30	600
10	7 hour	41	4100	32	640
11	8 hour	43	4300	34	680
12	9 hour	45	4500	35	700
13	10 hour	48	4800	37	740
14	11 hour	50	5000	39	780
15	12 hour	53	5300	41	820

#### TABLE- 3 : LETHAL DOSE TOXICITY:

NO.	NAME OF DISINFECTANT	TOXICITY DOSE	TIME IN	NO OF FISH
			HR.	LEFT
1	Leaves of acacia nilotica(juice	30ml-3000ml	96 hr	04
	form)			

#### 7. CONCLUSION:

From the above discussion it is conclude that, the natural disinfectants i.e. the acacia Nilotica plant are easily available in the Melghat area. In Melghat area, the people don't have knowledge about the disinfection method but, if they know the use of these plants, then they can easily use these plants for disinfection process. No skilled supervision or manpower is required if natural disinfectants can be used for the disinfection of water. In rural area, there is lack of water treatment

water. In rural area, there is lack of water treatment plant; therefore the lots of people are suffering from the waterborne diseases which are caused due to the pathogenic bacteria. If they know these plants can be used for killing of pathogenic bacteria. Then they can be easily used such plants without any difficulties these plants are available in large quantity so this method is economically cheap method.

## 8. LIMITATION

CFU/100

ML

160

80

40

02

The chlorinated water can be stored for 7 to 8 days but if natural disinfectants are used for disinfectants of water, then consume this water only in 2 days because if this water is stored for 7 to 8 days then the degradation of organic matter starts. This is the limitation of natural disinfectants.

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