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Bioload Reducing Potential of Neem

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Sewage is a guaranteed source of many pathogens which have the potential to initiate an epidemic outbreak. The classical approach of chlorination for disinfection of water bears reported and proven side effects. Neem (*Azadirachta indica* A. Juss) has been reported to contain potent antibacterial properties and is capable of inhibiting the growth of many harmful bacteria. In the present study, the active principal is isolated from neem and tested for its efficacy to reduce the bacterial bioload in a defined volume of sewage. The rate is compared with that of the pure form and with chlorine. Combined antibacterial efficacy of azadirachtin and chlorine was also analyzed. We conclude that azadirachtin present in neem has potent bactericidal properties against bacterial population in waste water and can be used in conjunction with chlorine for disinfection purpose.

Key words: Pathogens, Chlorination and Azadirachtin.

Water borne spread of pathogens is the most common method of pathogen dissemination. Sewage water, in many instances, after primary treatment like settling, is used for irrigation purposes in agricultural lands.

Application of untreated water in arid land, may lead to spread of pathogens. Sewage sludge contains pathogenic bacteria and if it is spread on arable land, there is risk of spreading diseases to people and animals. The reduction of pathogens in sludge depends on the treatment methods used to stabilize sludge in sewage treatment plants. (Leena Sahlstrom *et al*., 1995). Many studies have isolated majority of chemical compounds found in *A. indica*. (Ascher 1981, Schmutterer and Zebitz 1983). Of all the compounds isolated from the neem plant, azadirachtin is the most toxic. (Ascher 1981). Oil from the neem leaves, seeds and bark possesses a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms, including *M. tuberculosis* and streptomycin resistant strains (Chopra *et al*., 1952). *In vitro*, it inhibits *Vibrio cholerae*, *Klebsiella pneumoniae*, *M. tuberculosis* and *M. pyogenes* (Satyavati *et al*., 1976). In this investigation, azadirachtin – the active ingredient

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present in neem kernel is tested for its ability to reduce the bacterial population in a known volume of sewage sample. Azadirachtin – one of the active principal present in neem kernel is isolated in crude form and its antibacterial activity is determined. Commercial azadirachtin powder was also analyzed for its ability to reduce the bacterial load in sewage. The results were compared with the antibacterial effect of chlorine singly and in combination. Chlorine (Cl_2) has been widely used throughout the world as a chemical disinfectant, serving as the principal barrier to microbial contaminants in drinking water. The noteworthy biocidal attributes of chlorine have been somewhat offset by the formation of disinfectant by-products (DBPs) of public health concern during the chlorination process. In 1976, chloroform, a trihalomethane (THM) and a principal DBP, was shown to be carcinogenic in rodents. This prompted the U.S. Environmental Protection Agency (U.S. EPA) in 1979 to develop a drinking water rule that would provide guidance on the levels of THMs allowed in drinking water. Further concern was raised by epidemiology studies suggesting a weak association between the consumption of chlorinated drinking water and the occurrence of bladder, colon, and rectal cancer. In 1992 the U.S. EPA initiated a negotiated rulemaking to evaluate the need for additional controls for microbial pathogens and DBPs. The goal was to develop an approach that would reduce the level of exposure from disinfectants and DBPs without undermining the control of microbial pathogens. (Boorman 1999).

The investigations conducted will be greatly useful in reducing the detrimental effects caused by using chlorine as a disinfectant. A vast number of antibacterial studies using neem had been carried out earlier. But this study floods light on the efficacy of the antibacterial properties of neem on sewage born bacteria, which is perhaps a topic of novel application.

MATERIAL AND METHODS

Isolation of active principal from neem kernel (Kumar 2002)

Fresh ripened neem fruits were collected during the months of May to June from trees in Sourashtra College campus at Madurai. The fruits were squeezed to obtain the seeds. The seeds were dried under shade for a period of one week, after which they were break-opened to get the kernels. Neem kernel powder was obtained by pulverizing the kernels to a fine powder in a blender.

Collection of commercial azadirachtin

Commercial azadirachtin solution was purchased from Karna Oil Mills – Theni and used as the pure form of azadirachtin.

Preparation of Chlorine

2ppm of chlorine solution was prepared as hypochlorite solution and used as the commercial disinfectant solution.

Collection of sewage sample

The sewage body selected for study was the commercial sewage farm in Avaniapuram – Madurai. Samples were collected during morning hours and transported to the lab in an ice-cold container. The sample was stored for not more than 2 hours.

Preparation of azadirachtin extract

Commercial azadirachtin powder and the kernel powder were mixed with methanol in the ratio 3:1, kept overnight and stored as pure and crude forms of azadirachtin respectively.

Antibacterial study

A volume of 10ml of sewage was taken in 11 sterile tubes and were labeled 1, 2...11. The first tube served as control. To the second tube, 0.1ml of azadirachtin solution was added. To the next tube, 0.2ml of azadirachtin solution was added and allowed to stand. The procedure was followed by adding 0.3ml of extract to the fourth tube, 0.5ml of the extract to the fifth tube, etc., up to the eleventh tube, to which 1ml of solution was added. The tubes were allowed to stand for half-an-hour (Contact time).After the contact time, an aliquot of 1ml was inoculated in three nutrient agar plates (for triplicates) by pour plate technique, by pour plate technique. The plates were incubated at 37°c for 24 hours. After the period of incubation, the number of Colony Forming Units in the plates were counted, averaged and rounded. The results were tabulated indicating the concentration of azadirachtin and the number of Colony Forming Units in each concentration (Table 1). A graph was plotted with azadirachtin concentration in X-axis and the number of Colony Forming Units in Y-axis (Fig 1).

The procedure was repeated with crude extract of azadirachtin from neem kernel and chlorine individually and in combined fashion with chlorine in a constant volume and with increasing concentrations of pure azadirachtin and the results were tabulated (Tables 2,3 and 4). The corresponding graphs were plotted (Fig 2,3 and4). The results are discussed.

RESULTS AND DISCUSSION

Chlorine has long been used as a commercial disinfectant to reduce bacterial load. But addition of chlorine results in the production of Tri Halo Methane (THMs), which are carcinogenic. It had already been reported that neem kernels have antibacterial principals and

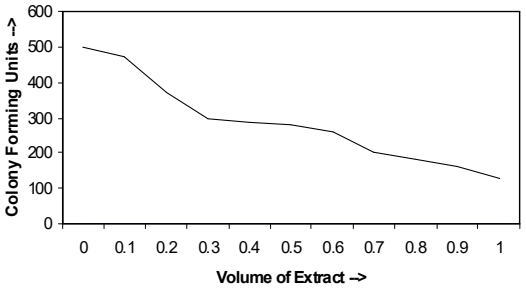

Fig. 1. Antibacterial effect of commercial Azadirachtin on bacteria

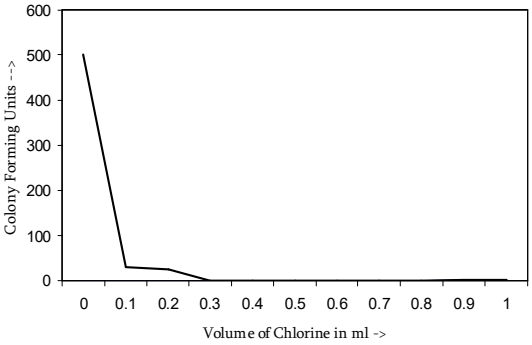

Fig. 3. Antibacterial effect of Chlorine on bacteria

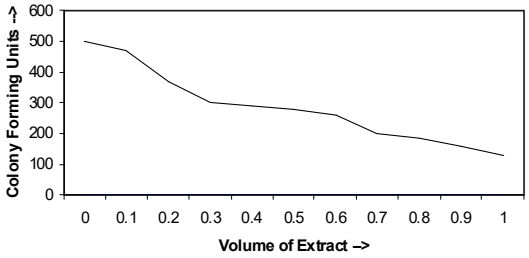

Fig. 2. Antibacterial effect of crude Azadirachtin on bacteria

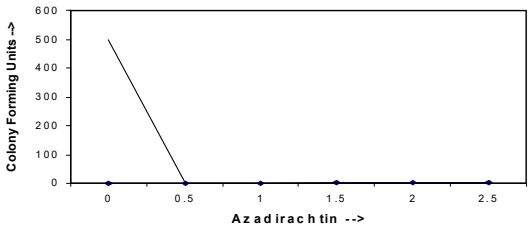

Fig. 4. Antibacterial effect of Azadirachtin + 0.05ml chlorine on bacteria

Table 1. Antibacterial activity of Commercial Azadirachtin

S. No.	Volume of Sewage (ml)	Volume of Azadirachtin solution (ml)	No. of Colony Forming Units
1.	10	0	500
2.	10	0.1	400
3.	10	0.2	300
4.	10	0.3	230
5.	10	0.4	190
6.	10	0.5	160
7.	10	0.6	142
8.	10	0.7	132
9.	10	0.8	93
10.	10	0.9	70
11.	10	1.0	30

Table 2. Antibacterial activity of crude Azadirachtin

S. No.	Volume of Sewage (ml)	Volume of Extract (ml)	No. of Colony Forming Units
1.	10	0	500
2.	10	0.1	470
3.	10	0.2	370
4.	10	0.3	300
5.	10	0.4	289
6.	10	0.5	280
7.	10	0.6	260
8.	10	0.7	200
9.	10	0.8	185
10.	10	0.9	160
11.	10	1.0	130

Table 3. Antibacterial activity of Chlorine

S. No.	Volume of Sewage (ml)	Volume of Chlorine (ml)	No. of Colony Forming Units
1.	10	0	500
2.	10	0.1	30
3.	10	0.2	25
4.	10	0.3	0
5.	10	0.4	0
6.	10	0.5	0
7.	10	0.6	0
8.	10	0.7	0
9.	10	0.8	0
10.	10	0.9	0
11.	10	1.0	0

in this study, the antibacterial potential of neem kernel extract is tested to reduce the bacterial count in sewage. The results of the study indicate that neem kernels contain azadirachtin and has good potential to reduce bacterial population.

Table 1 reveals that countable colonies appeared at an azadirachtin concentration of 0.2ml, whereas in the case of crude azadirachtin (kernel extract), countable colonies appeared only at a concentration of 0.3ml. This reflects that as the concentration or purity of the antibacterial principal increases, there is a reduction in the colony count. With chlorine (Table 3), countable colonoies appeared at a volume of 0.1ml, which was close to the TFTC (Too Few To Count) value. This proves that chlorine has potent bactericidal property. When combined with 0.05ml chlorine, no colonies were obtained even at the initial test volume of 0.5ml of azadirachtin. This states that addition of azadirachtin to chlorine, improves the bactericidal property of the latter. This finding may reduce the volume of chlorine applied for disinfection, which may potentially reduce the risk of side effects associated with chlorination.

Table 4. Antibacterial effect of Commercial Azadirachtin + Chlorine

S. No.	Volume of Sewage ml	Volume of Chlorine ml	Volume of Azadirachtin solution ml	No. of Colony Forming Units
1.	10	0	0	500
2.	10	.05	0.5	0
3.	10	.05	1	0
4.	10	.05	1.5	0
5.	10	.05	2	0
6.	10	.05	2.5	0

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