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RESEARCH ARTICLE

Antimicrobial activity of Zoysia grass (Turf Grass/Lawn Grass) on Total Coliform: A low cost potential Water disinfectants

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Abstract

Water is the universal component, needed by human beings everyday for various purposes like drinking, washing, bathing, in agricultural lands etc. Burgeoning population increases the demand for industrial establishments to meet human needs, which in turn has created many environment problems, including water pollution. Microbial contamination of fresh water bodies is also a common problem, as water from most of the water bodies requires disinfectant, particularly when water is used for human consumption. Presence of particular type of microbes decides which type of disinfectant will be suitable for eliminating microbial contamination. The water disinfection problem is large and complex in developing countries. Among the various methods of disinfection, chlorine continues to be one of the favorite disinfectants, worldwide. However, it had been shown that using chlorine might produce various deleterious compounds such as Trihalomethanes (THM's), which were reported to be carcinogenic in nature. The present paper mainly expresses the potential of Emerald zoysia grass in treating water contaminated with coliforms. Further, techno-economical feasibility of its use as a substitute for chlorine in water disinfection is being looked into.

INTRODUCTION

Water constitutes one of the important physical environments of human being & has direct effect on itself. Water supplied should be aesthetically clean & biologically safe (Somami et al., 2011). The World Health Organization reports (WHO, 2014) that 1.6 million people die every year from diarrheal diseases attributable to lack of access to safe drinking water and basic sanitation. 90% of these are children under the age of five, mostly in developing countries. Therefore, for several decades in developed countries; the stress is given on improvement of technology for purification of drinking water. This is primarily due to new findings on the effects of various harmful and dangerous substances that are present in natural waters or are formed in the water treatment process, especially with the use of oxidizing agents, primarily chlorine (Pavlovic et al., 2014). Apart from increased concentrations of physico-chemical constituents in raw water, new rising problem is bacterial, i.e. microbiological water pollution. In water treatment processing, process of disinfection has very important role (Pavlovic et al., 2014).

In water treatment applications, chlorine used for disinfection can react with organic material to produce chloro-organic compounds that are highly carcinogenic (Kool et al., 1985; Dunlop, 2002). In addition, some pathogens such as viruses, certain bacteria such as Legionella, protozoans such as Cryptosporidium and Giardia lamblia cysts have been known to be resistant to chlorine disinfection (Pontis, 1990; Regli, 1992). Other treatment alternatives such as ozonation and irradiation using germicidal lamps (254 nm) have their own troubles and boundaries, such as lack of residual effect (Masschelin, 2002) and generation of small colony variants (Robertson, 2005) for the latter and production of toxic disinfection byproducts for the former (Huang, 2005). Therefore, to withstand all this problems and to combat future problems it becomes important to find a further low cost solution.

Grass is the world's most ubiquitous plant, an incredible survivor and is virtually indestructible. Most grasses are annual or perennial herbs with fibrous roots and, often, rhizomes (Dashora and Gosavi, 2013). Therefore, from availability point of view, they are copious.

Zoysia (Lawn grass, Turf grass) comes under the genus of creeping grasses. It is widespread across much of Asia as well as Australia and various islands in the Pacific. These species, commonly called zoysia or zoysia grass, are found in coastal areas or grasslands (Wikipedia). Different species of this grass ranges from the very fine-leaved Korean velvet grass (Zoysia tenuifolia), used to a limited extent as an unmowed, meadow-like ground cover, to Japanese lawn grass (Z.japonica), a relatively coarse bladed turf grass used in lawns. Next, include the fine-bladed Manila grass or Korean lawn grass (Z. matrella) that is used for low-input lawns in Asia, and the minor use, salt tolerant Z. sinica and Z. machrostaycha (Gibeault, 2003). Zoysia emerald is a hybrid of Zoysia japonica and Zoysia matrella and it is naturalized in India (Wikipedia). These grasses are mostly used for aesthetic purposes and for maintaining their evenness, it is used to cut regularly so it is easily available to be work with.

Looking towards the chemical properties of the zoysia grass it has been already reported that chlorogenic acid and a group of more than15 flavonoids that gave UV spectra, indicating all were luteolin-glycosides (see structures in Fig. 1). Major constituents in zoysia grass were luteolin glucosylarabinoside, luteolin-diarabinoside, and methoxy-luteolin-diarabinoside, as determined by UV, hydrolysis, and Mass Spectrometry methods. Percentage weight of cholinergic acid, total flavonoids and phenolics are found to be 0.063-0.008, 0.581-0.071 and 0.644-0.079 respectively in Emerald zoysia (Jhonsen et al., 2002)

However, chlorogenic acid was higher in centipede grass than zoysia grass, but both grasses were highly toxic to Fall army worm. This would indicate that other chemical compounds are probably involved in zoysia grass, or the types of luteolins found in zoysia grass may be more toxic to Fall army worm (Jhonsen et al., 2002)

Thus, it is important to search a technique or technology through which microbiologically clean water for drinking, for food and industries can be available with fewer disadvantages, which we are facing with current disinfectants (Cavanagh, 1992; Krasner et al., 2006).

2. Materials and Methods

2.1. Water Sample Collection

Water samples were collected from different wells, properly preserved and transported to laboratory for analysis as per Standard methods, APHA (American Public Health Association, 1995).

2.2. Zoysia grass Collection

Zoysia emerald grass (without root) was collected from the lawn existing within the Institute premises. Collected grass was washed, dried, grinded and sieved into powder and was kept in airtight bottles for further use.

3. Procedure

All the glassware were sterilized in an autoclave at 15lbs (at 120°C) for 15 minutes before the start of work. Selective media plates i.e. M-endo plates were prepared for total coliforms determination. Microbial analysis of water samples was done using membrane filtration technique (MFT) (APHA, 1995).

Experiment 1

Extract from Zoysia grass was prepared by mixing 10gms of powdered grass in 90ml sterilized distilled water and keeping it in an incubator-shaker for 90mins. 100ml sample was processed through MFT. Later, 1ml of the above said extract was poured on sample plates over the filter paper, which are processed earlier. Control was also prepared with distilled water. All the plates including control were kept in an incubator at 37±5°C and readings were noted for 24 hrs and 48 hrs respectively to see the microbial growth.

Experiment 2

Observing the plates at different incubation period (Time)

1. After 1 hour
2. After 2 hour
3. After 5 hour
4. After 10 hour

Procedure for analysis of sample was same as mentioned in experiment 1. Only observation was done at different incubation time. The sample, which was labeled as "After 5hrs", was also observed after10hrs and the result was same. Later on, all the treated plates were also kept for 24hrs, the result was observed, and it was showing no growth.

Experiment 3

Observing the plates (after 24hrs) at different retention period after mixing the sample and Zoysia emerald.

1. After 1 hour
2. After 2 hour
3. After 5 hour
4. After 10 hour

A set of four conical flasks was prepared and labeled as 1hr, 2hrs, 5hrs, and 10hrs respectively.100ml sample was taken in each conical flask and, 2g of Zoysia emerald grass powder was mixed in it and then it was kept in an incubator shaker. After 1hr, sample that was labeled as 1hr was taken out and processed by the same method as mentioned above in experiment 1 by MFT except that no further addition of Zoysia emerald was done . Same procedure was repeated for the sample labeled as 2hr, 5hr and 10hr respectively i.e. after each successive period of time sample was taken out, processed and observation was done after 24 hrs. It was found that no Coliform was present in the samples, which were mixed with Zoysia emerald powder, whereas, a well-developed growth of Coliform was observed in untreated sample.

4. Results and Discussions

The first step of the work was to ensure whether the present sample contained Coliform or not. For this, basic tests (Bartram and Balance, 1996) of broad classification by Gram-staining followed by Oxidase test and Lactose fermentation test. Later on, Microscopic analysis was performed. Finally, Plating was done on selective media; where in M-endo Himedia was used. Results obtained from various tests for the presence of coliform are given in Table 1.

After the initial test, experiment was done to check the antimicrobial activity of the said grass. Total count of the plates decreased considerably after treatment. Results of the various samples with and without treatment are shown in Table 2.

Perusal of Table 2 indicates that Zoysia grass has shown greater potential of antimicrobial activity. As many as 8 out of 10 samples have shown complete removal of coliforms, whereas rest 2 samples (Sample 3 & 6) have shown negligible presence of only one coliform. This incomplete removal may be an indication that the procedure needs to be further optimized/modified for complete use of the antimicrobial activity of the grass. One such modification may be conversion of these grasses as activated carbon and then use it for treatment; it may or may not be increase/decrease the activity but it will surely add up the cost and energy to the procedure. Using more and more sustainable processes and amplifying their activity is the need of the hour. Apart from thinking about sustainable development, other points, which should be considered, are cost and efficacy and Zoysia can meet these challenges in future.

The microbial disinfection efficiency of Zoysia emerald grass is depicted in the figures 5 which shows the samples after treated with the Zoysia grass, no growth of coliform was observed on plates. A plate with distilled water has also done and taken as control, figure 2.

Experiments were also done to check the efficiency of the grass to control the growth of the coliform at different incubation time. This has also given a positive result as at different time the effect of Zoysia emerald is same as that in after 24hrs as sheen in figure 6. In addition, it has been observed that at different retention time of the sample with Zoysia emerald had produced effective result as shown in figure 7.

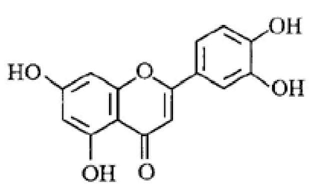
Table 1: Tests for Presence of Coliforms

Tests	Results
Gram Staining	Negative
Oxidase	Negative
Lactose fermentation with production of acid and gas at 24 hrs & 48 hrs	Positive
Microscopic analysis	Rod shaped
Plating on selective media (M-endo)	Appearance - Green metallic sheen

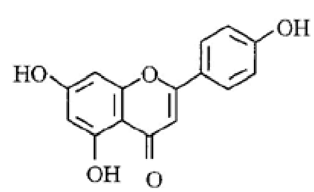
Table 2: Presence of Total Coliforms in Untreated and Treated Water Samples

Sample No.	Total Coliforms (CFU/100ml)	
	Untreated Samples	After Treatment with Zoysia grass
1	40	Nil
2	60	Nil
3	TNTC	1
4	TNTC	Nil
5	330	Nil
6	TNTC	1
7	290	Nil
8	TNTC	Nil
9	TNTC	Nil
10	296	Nil

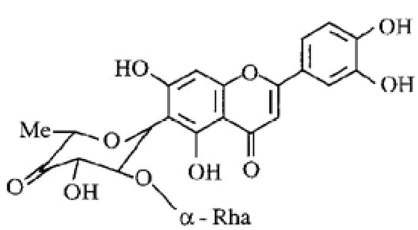
CFU- Colony Forming Unit/100mL; TNTC- Too Numerous To Count



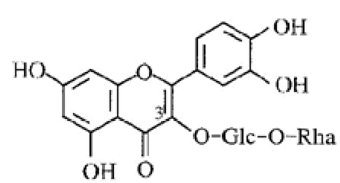
Luteolin



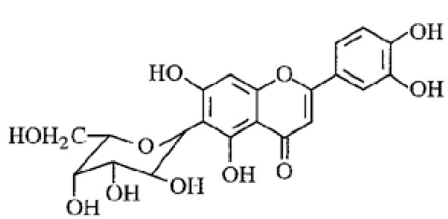
Apigenin

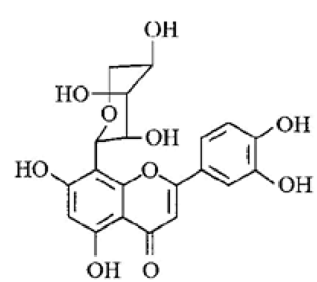


Maysin



Rutin





6-C-ß-D-glucopyranosyl-luteolin

Isoorientin

8-C-ß-L-Arabinopyranosyl-luteolin

Figure 1: Chemical structures of the major polyphenols and flavonoids found in certain turf grass species (Jhonsen et al., 2002).

Figure 2: Control (plated with distilled water)

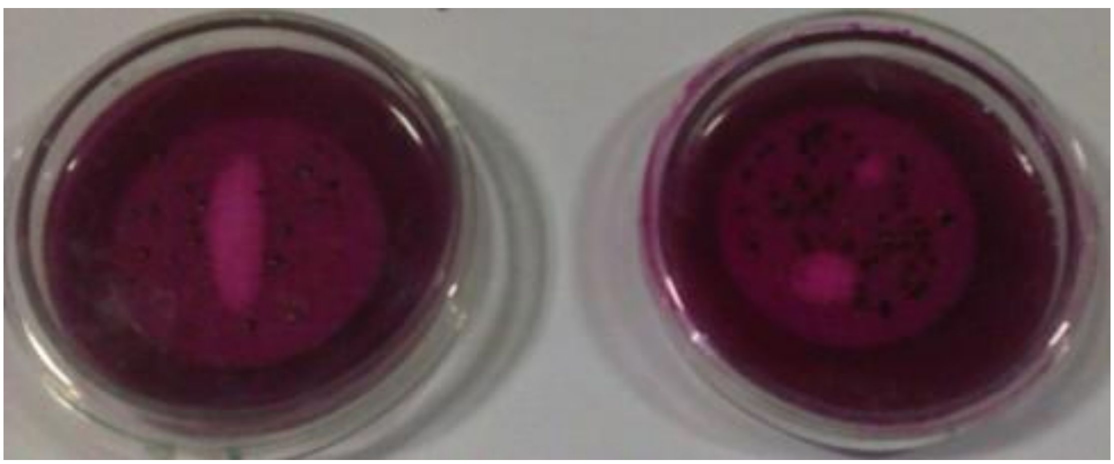


Figure 3: Sample 3 and 10 without treatment after 24 hours

Figure 4: Sample 3 and 10 without treatment after 48 hours

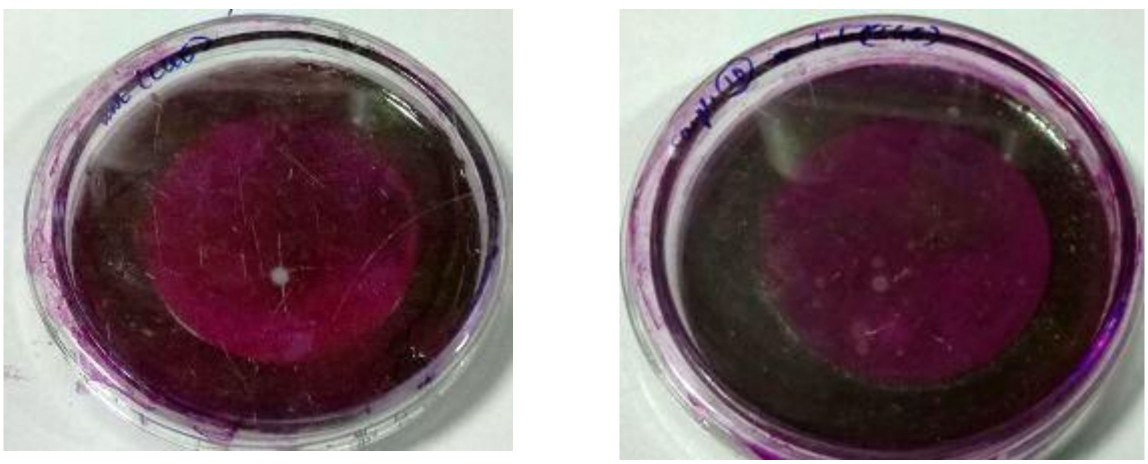
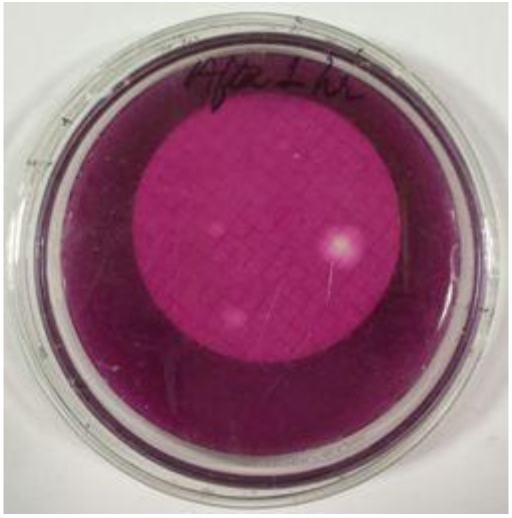
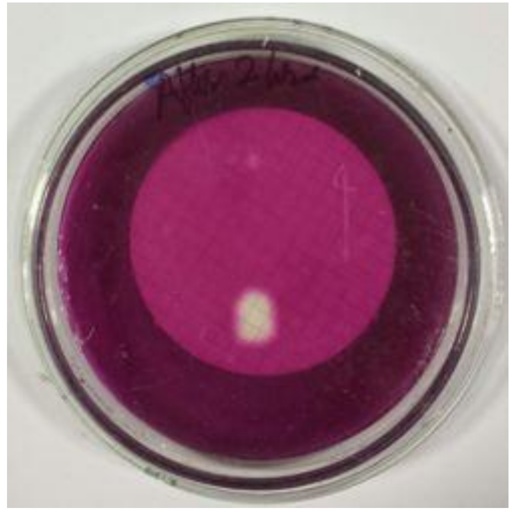


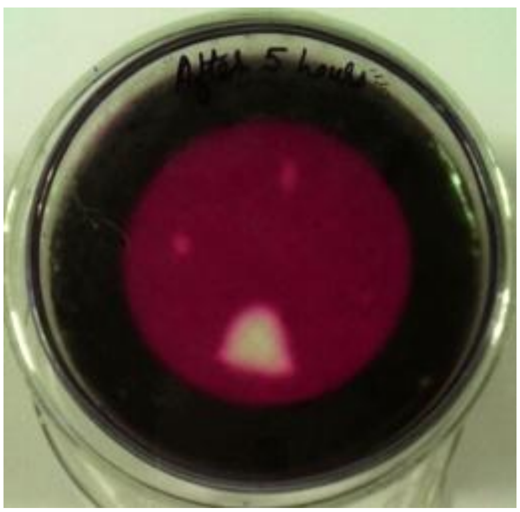
Figure 5: Sample 3 and 10 after treatment with Zoysia grass (Lawn grass)



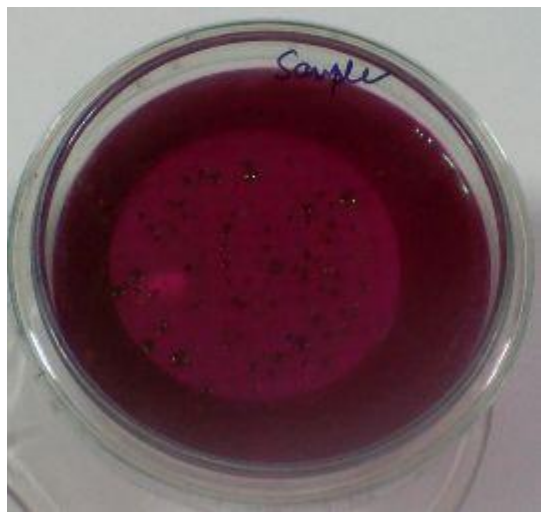
a. after 1 hour



b. after 2 hours



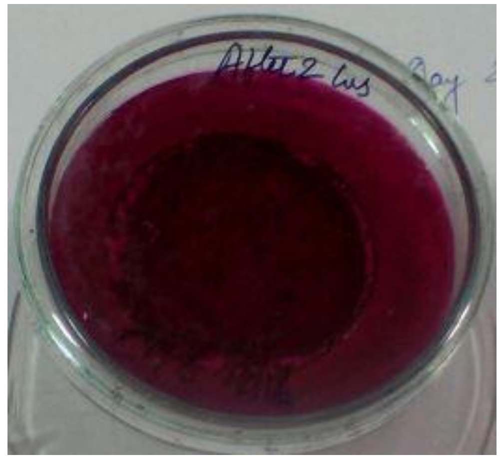
c. after 5 hours



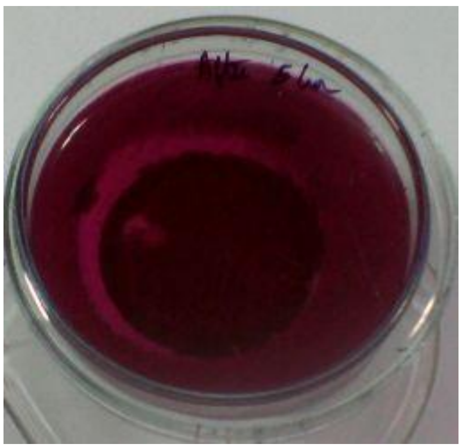
d. Growth in untreated sample

Figure 6: Sample observed at different incubation time after treatment with Zoysia emerald i.e. showing no growth (a) after 1hour, (b) after 2 hour, (c) after 5hour but an untreated sample (d) named as “Sample” showed full growth.

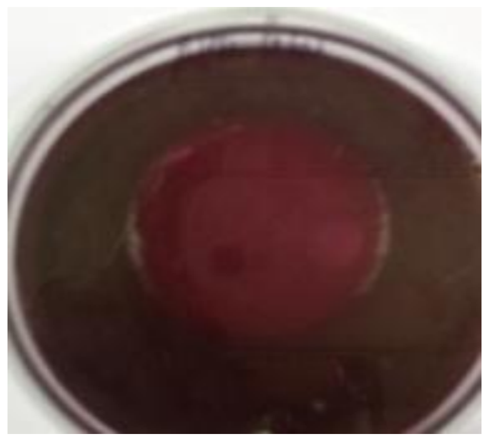
a. after 1 hour



b. after 2 hours



c. after 5 hours



d. after 10 hours

e. Growth in untreated Sample

Figure 7: showing no growth when sample was treated with Zoysia emerald kept at different retention time i.e. (a) kept for 1hour, (b) kept for 2 hours, (c) kept for 5 hours, (d) kept for 10 hours, then processed and observed after 24hrs. However, an untreated sample (e) showing full of colonies after 24hrs was labeled as “Sample”.

5. Conclusion

India is well flourished with the enormous natural flora and this flora has various advantageous properties, which are still not explored. The study shows simple and effective method to disinfect water at preliminary stage with the help of this natural flora. The current work leads to the conclusion that Zoysia emerald/turf grass has significant potential of antimicrobial activity. Therefore, it can act as an alternative to the present techniques of water disinfection in near future. Most of the gram-negative bacteria have become resistant to antibiotics (Basile et al., 2000). Hence, it can also serve as a boon for Pharma companies in preparing antibiotics, keeping its pros and cons in mind. The proposed method is simple and cost effective, more research and studies is underway on its various aspects for its safe use as low cost water disinfectant.

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