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Harbouring of Pathogenic Microorganisms by Aquatic Weed, *Eichhornia crassipes* in its Rhizosphere

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Abstract: Alappuzha district, Kerala state, India has a wide network of rivers, canals, backwaters and most of the water resources are highly contaminated due to the disposal of organic waste, fertilizer residues and run offs from agricultural fields into water bodies. As a result of this water bodies have become a congenial breeding ground for water borne vectors like mosquitoes and pathogenic microorganisms. Availability of safe drinking water is also a serious concern and water borne diseases constitute one of the major public health hazards in this region. In the present study, water samples from (rhizosphere region of aquatic weed, *Eichhornia crassipes*) five different locations of Cherthala taluk were collected and performed a comparative study on the microbial population and for the presence of *E. coli*, *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Salmonella typhi.* All the water samples were found contaminated with *E. coli*, *V. cholera* and *S. typhi. Vibrio parahaemolyticus* was found only in three samples. It was also observed that these pathogenic microbes were present very near to the rhizosphere region of *E. crassipes.* The presence of amino acids glutamic acid, hydroxyl proline, leucine and threonine were found from the rhizosphere regions and these amino acids attracted the pathogenic microbes towards *E. crassipes.*

Keywords : amino acids, *Eichhornia crassipes,* root exudates, *Salmonella typhi, Vibrio cholerae.*

INTRODUCTION

Water has been of great importance to human beings and other organisms of the environment for sustenance of life and maintaining the balance of the nature, hence water is called as the “life blood of the earth”.[1] Microorganisms can exist naturally or can occur as a result of contamination from human or animal waste. The three main types of microorganisms that found in drinking water are bacteria, viruses and protozoa. The consumption of drinking water containing enteric pathogenic bacteria has been linked to illnesses commonly present as gastrointestinal-related symptoms, such as diarrhorea and nausea. Faecal indicators, such as *E. coli*, are the best available surrogates for predicting the presence of such organisms. Its presence in water indicates not only recent fecal contamination of the water but also the possible presence of intestinal disease causing bacteria, viruses, and protozoa. *E. coli* is the only member of the total coli form group that is found exclusively in faeces, other members of the group are found naturally in water, soil, and vegetation, as well as in faeces. *S. typhi* is one of the major causes of food and water borne gastroenteritis in human[2] and remains an important health problem worldwide. The World Health Organization estimates 16 million new cases and 600,000 deaths of typhoid fever were estimated each year.[3] *Vibrio cholerae*, a member of the family Vibrionaceae, is a facultative anaerobic, gram-negative, non-spore-forming curved rod, about 1.4 - 2.6 mm

long, capable of respiratory and fermentative metabolism; it is well defined on the basis of biochemical tests and DNA homology studies[4] is also an important pathogen that spreads through contaminated water.

Water hyacinth (*Eichhornia crassipes*) is an aquatic plant which can flourish and reproduce floating freely on the surface of water or it can also be anchored in mud.[5] It is a perennial weed, form dense rafts in the water and mud. Some of the principal problems are its interference with navigation, water flow, and the recreational use of aquatic systems, as well as the risk it poses of mechanical damage to hydroelectric systems. It is also responsible for drastic changes in the plant and animal communities of freshwater environments and acts as an agent for the spread of serious diseases in tropical countries.[6] Therefore for the last several years, many investigators have directed their research to the microbial ecosystem utilising the micronutrients of the root exudates. In this study heterophilic plate count, amino acid profile of rhizosphere root exudates of *E. crassipes* was screened and analyzed for role in the chemotaxis of pathogenic microbes towards its rhizosphere.

EXPERIMENTAL

CHEMICALS

Tryptone Glucose Extract Agar, selenite enrichment broth, alkaline peptone water, Eosin Methylene Blue (EMB) agar, Bismuth Sulfite (BS) Agar, Thiosulphate Citrate Bile salt Sucrose (TCBS) agar, standard amino acids were procured from HiMedia Laboratories Pvt. Limited, Mumbai, India.

SAMPLE COLLECTION

Fifty ml of water samples were collected from the rhizosphere region of *E. crassipes* from five different locations, approximately one kilo meter apart in Cherthala taluk. The pH and temperature of the samples were monitored at the time of collection.

HETEROPHILIC PLATE COUNT

Heterophilic plate count (HPC) serves as an indicator of general microbial population and samples to be analysed for quantitative bacterial analysis were plated on Tryptone Glucose Extract Agar.[7] The plates were incubated at 37°C for 24 hours and the total plate count was performed. The experiment was done in triplicates and the mean values were expressed.

DETECTION OF PATHOGENIC MICROORGANISMS

The presence of *E. coli* was detected by plating the samples on Eosin Methylene Blue (EMB) agar plates. *Salmonella typhi* was isolated by inoculating one ml of water sample into 10 ml of selenite enrichment broth and incubated at 37 ^{o}C for 12-18 hours. Swabs from the selenite broth were streaked on to Bismuth Sulfite (BS) Agar plates, and further incubated at 37^{o}C for 24 - 48 hours.

Water samples suspected to contain *V. cholerae* and *V. parahaemolyticus* were enriched by adding 100 ml water sample in 200 ml of double strength alkaline peptone water (pH 8.6) at 37 ^{o}C for 24 hours and swabs from the alkaline peptone water was streaked on to Thiosulphate Citrate Bile salt Sucrose (TCBS) agar and further incubated at 37^{o}C for 24 - 48 hours.[8]

COLLECTION OF ROOT EXUDATES

Exudates were collected from *E. crassipes* roots by covering the plant roots with sterile tissue papers and the roots were packed in test tubes. The tissue papers were replaced every two days with fresh sterile papers and this process was continued for two weeks to get a detectable amount of amino acids. The tissue papers were removed from the root surface and stored in 100 % alcohol, which was then concentrated under vacuum and used for paper chromatography to detect amino acids from the root exudates.

ANALYSIS OF ROOT EXUDATES

The amino acids from the root exudates were analysed using paper chromatography. The following amino acids were used as reference.[9] They are γ-aminobutyric acid, alanine, arginine, asparagines, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, hydroxyl proline, serine, threonine, tryptophan, tyrosine and valine. Out of these amino acids phenylalanine and tyrosine were dissolved in 0.05 normal (N) HCl and amino acid tryptophan was dissolved in 0.05 N NaOH. The rest of the amino acids were dissolved in distilled water at a concentration of 1 mg/ml. Ninhydrin (0.33 %) prepared in water was used as detecting reagent.[10]

For mobile phase n-butanol, acetic acid and water in the ratio 12: 3: 5 was poured into the chromatography chamber. The whatman No.1 sheet was cut according to the size of glass chamber and the test samples and the standards amino acid solution were spotted in the whatman No.1 filter paper and placed inside the chromatographic chamber. After four hours, the paper was taken out, air-dried, sprayed with ninhydrin solution and placed in an oven at 100 ^{o}C for 10 minutes.

The relative front (R_f) value was calculated by using the formula

$$R_f = \frac{\text{Distance (cm) moved by the solute from the origin}}{\text{Distance (cm) moved by the solvent from the origin}}$$

RESULTS AND DISCUSSION

The hetrophilic plate count of samples collected from five different locations were in the range of 61×10^4 to 77×10^4 CFU/ ml (Table 1). The pH and temperature of the water samples collected ranged from 6.4 to 6.7 and 29 to 31ºC respectively (Table 1). *E. crassipes* prefers a pH range between 6 and 8 for its growth. The pH of water samples collected from the rhizospheric region of *E. crassipes* were in the range of 6.4 and 6.7 and this also favoured the growth of microorganisms. Nebel[11] observed the optimal conditions for the survival and growth of toxigenic *V. cholerae* 01 in aquatic environments rich in nutrients are moderate salinity, warm temperatures and neutral or slightly alkaline pH. These conditions are typical of estuaries and coastal swamps in equatorial, tropical, and subtropical regions, where many species of phytoplankton and zooplankton, fish, mollusks, and crustaceans thrive.[12]

Table: 1. Analysis of rhizosphere water samples

Water samples	Total bacterial count (CFU/ml)	pH	Temperature (ºC)	Detected amino acids
Sample - I	61 x 10^4	6.4	30	Hydroxyl proline, Leucine and Threonine
Sample - II	77 x 10^4	6.6	30	Glutamic acid, Hydroxyl proline, Leucine and Threonine
Sample - III	74 x 10^4	6.8	29	Hydroxyl proline and Threonine
Sample - IV	68 x 10^4	6.7	30	Glutamic acid, Leucine and Threonine
Sample - V	63 x 10^4	6.6	30	Glutamic acid, Hydroxyl proline, Leucine and Threonine

The amino acid analyses in the rhizospheric root exudates of *E. crassipes* revealed the presence of amino acids such as glutamic acid, hydroxy proline, leucine and threonine (Table 1). The pathogenic micro organisms such as *E. coli, S. typhi, V. cholerae* and *Vibrio parahaemolyticus* were detected from the different samples (Table 2).

S. typhi, V. cholerae and *E. coli* were found in all the samples collected. *V. parahaemolyticus* was found only in sample II, IV and V. The presence of *S. typhi, V. cholerae* and *E. coli* from Cherthala region were previously reported by Chandran et al.[13] The heavy heterophilic bacterial count in the rhizosphere region was a clear indication of chemotaxis of microbes towards rhizosphere region of *E. crassipes*. This was attributed to the presence of different amino acids found in the root exudates secreted by *E. crassipes.* The toxigenic *V. cholerae* 01 biotype El Tor has been isolated from macrophytes in both seawater and fresh water.[14-16] Zhao-Dajun and Zheng-Shizhang[17] reported amino acids, methionine, gamma amino butyric acid, glycine, alanine, aspartate, serine, valine, and leucine from the root exudates of *E. crassipes* and these amino acids were found attracting *Enterobacter* sp. F-2.

Table 2: Presence of pathogenic microorganism in water samples

Water samples	*V. cholerae*	*S. typhi*	*E. coli*	*V. parahaemolyticus*
Sample - I	+	+	+	-
Sample - II	+	+	+	+
Sample - III	+	+	+	-
Sample - IV	+	+	+	+
Sample - V	+	+	+	+

'+' Presence and '- ' absence

Islam et al.[15] have suggested that aquatic plants could be environmental reservoirs of the microbe through either a nonspecific association or a commensal relationship.[16] The stems and roots of the water hyacinth are often harbour microorganisms such as the nitrogen fixing *Azotobacter chroococcum*[6] and vertebrates and invertebrates with which it may not infrequently form symbiotic relationships with amphibians, bony fish, snakes, rodents, annelids, and arachnids and other arthropods.[18,19]

CONCLUSIONS

The present study on the detection of amino acids and co-existence of pathogenic microorganism near the rhizosphere region of *E. crassipes* from the five different locations in Cherthala taluk revealed the presence of pathogenic microorganisms such as, *E. coli, S. typhi, V. cholerae* and *V. parahaemolyticus*. Moreover the existence of these pathogenic microorganisms might be due to the presence of amino acids secreted and the favorable microenvironment exerted by *E. crassipes*. These pathogens are the causative agents of waterborne illness and this warrants the immediate attention to alleviate the menace of *E. crassipes.*

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