ANTHELMINTIC EFFECTS OF EXTRACTS OF SOME INDIGENOUSLY ISOLATED CULTURABLE CYANOBACTERIAL SPECIES

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Summary

In the present study methanolic and aqueous extracts of some indigenously isolated culturable cyanobacterial species especially from various sites of North Maharashtra region in India were tested for anthelmintic activities. After collection of samples, isolation of cyanobacterial pure cultures was done under laboratory conditions and identified by microscopic methods. Culturability of isolated strains was assessed under optimized conditions in BG11 medium. Pure cultures were preserved under cyanobacteriostatic phase.

Culturable strains were selected for cultivation of suitable cell mass. Methanolic and aqueous extracts were obtained from dried cell mass at 40°c temperature. These extracts were used for anthelmintic activities against test organism *Pheretima posthuma*. The anthelmintic assay was carried out in triplicates and the interpretation of results was done in terms of paralysis and death time in min. Some species were found to be a good source for an anthelmintic activity.

Key words: Cyanobacteria, Isolation, Anthelmintic, Methanolic, Aqueous, Extracts,

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Introduction

Among the oldest organisms on earth, dating back in the fossil record to nearly 3.5 billion years ago, the cyanobacteria ("blue-green algae") have evolved to produces an impressive array of biologically active compounds[1]. These are gram-negative photoautotrophic prokaryotes[2-3] occur in wide range of habitats[4] from ice fields to hot springs and deserts[5], freshwater, marine and terrestrial habitats[6]. Cyanobacterial habitats may be characterized by varying light intensity, temperature, water availability, nutrient availability, and ion levels[7].

Bioactive metabolites with pharmaceutical potential have been isolated from a variety of cyanobacteria[8]. Since cyanobacteria are known for their potential to produce secondary metabolites with a wide variety of bioactivities, they have received much attention as rich sources of novel bioactive compounds applicable to the production of medicines and agricultural chemicals[9]. Of the number of cyanobacterial species, some have been shown to possess antiplasmodial activity[10].

Helminth infections are among the most common infections in humans, affecting a large population of the world, particularly in tropical and subtropical areas[11] and pose a great threat to health and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia[12]. Number of survey results[13-14] indicated that, the intestinal helminth infections are prevalent in various areas. The three major Soil-Transmitted Helminths (STH's), *Ascaris lumbricoides* (roundworm)*, Necator americanus/Ancylostoma duodenale* (the hookworms) *Trichuris trichiura* (whipworm) are amongst the most prevalent parasites[15] worldwide. A number of chemicals had been effectively controlling/eliminating the worms, but increasing prevalence of anthelmintic resistance in these organisms[16-17] has encouraged research into alternative strategies for the control of these parasites. Hence, in the present work extracts of indigenous isolates of cyanobacterial species were tested for anthelmintic properties.

Material and Methods

Cyanobacterial Sample collection: -

Different sites were selected for sample collection, which probably dominates the cyanobacterial species, which include Tapi river sites, canals, Saatpuda regions, and farm fields, from North Maharashtra region in India. Most of the sampling was carried out from July to November, as sufficient growth has occurred during this period.

During sampling, the visible colonies, layers/mats, from different streams, ponds, and lakes especially from freshwater and terrestrial areas were selected. Solid samples were taken in sterilized bags whereas liquid samples in small white (type one glass) bottles and were transported to the laboratory on the same day[18-19].

Isolation and Identification of cyanobacterial cultures: -

Immediately after bringing to the laboratory, solid samples were washed by distilled water and the inoculums were prepared aseptically. For inoculum preparation, both the liquid and solid samples were diluted as per the requirement by serial dilution. For isolation and purification purpose the inoculums were processed by streak plate technique on solid BG-11 medium[20].

Repeated subcultures from solid to solid and/or from solid to liquid media were made till pure cultures were obtained. The growth conditions include temperature $25^0C \pm 2^0C$ and light intensity of about 2000-3000 lux[21]. After incubation for two to three weeks visible growth was observed in the form of colonies or lawn, if the species is filamentous. Moreover, the filamentous motile forms were satisfactorily isolated by their phototactic responses[22].

The isolated pure cultures were identified by using microscopic method. The morphological parameters such as cell shape, width, and length of intercalary cells; presence or absence of constriction at the cross wall, and of a sheath, color of the sheath, nature of trichomes, and filaments, presence or absence of heterocyst, width and length of heterocyst were taken into consideration during the identification of taxa. Identification of morphospecies was made using the major floras to cyanobacteria[23-24], as well as descriptions in the research papers based on biodiversity[25], studies were used for identification of cyanobacterial morphotypes[26].

Sample preparation:-

Isolated cyanobacterial cultures were cultivated in 250ml. Erlenmeyer flask containing 150ml. BG.11 liquid medium[21] in stationary conditions for 30 days at $25^0C \pm 2^0C$ and under illumination of white continuous light[22]. After satisfactory growth, the cyanobacterial mass was collected by suitable method i.e. either filtration or centrifugation[27]. The mass was dried at room temperature and triturated.

Extraction using maceration technique was carried by methanol, propanol and distilled water; each solvent was treated twice. The supernatants were collected by centrifugation and evaporated at 40°C to obtain the final extract as a sample and stored at low temperature till its further use[28-29].

Anthelmintic activity:

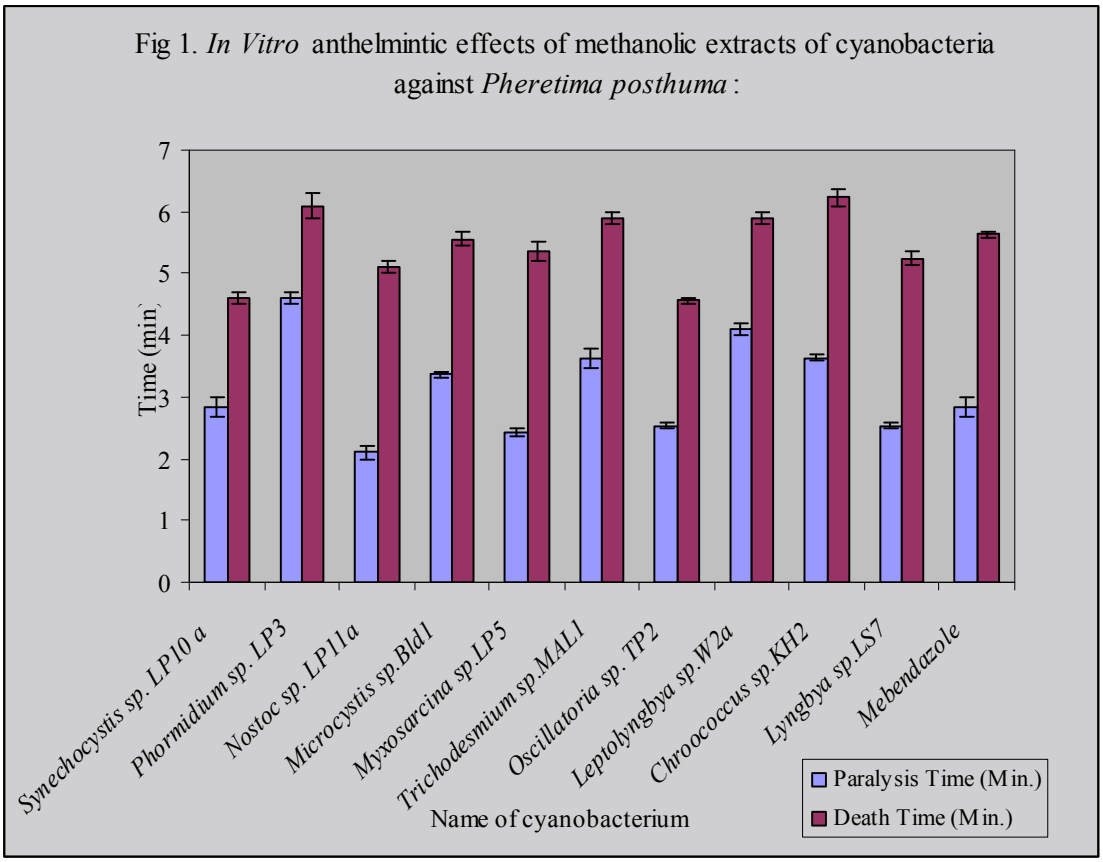
The anthelmintic activity was carried out on adult Indian earthworms, *Pheretima posthuma* in view of its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings[30-31]. Group of test organism each containing six earthworms[32]of approximately equal sizes were released into 10 ml of desired preparation. The dose suspensions were prepared using carboxymethylcellulose sodium (1% CMC), which is nontoxic and nonirritant used in oral and other formulations[33-34]. Each group was treated with one of the following, vehicle (1% CMC in normal saline), saturated solution of each cyanobacterial extract viz methanolic and aqueous, prepared in normal saline containing 1% CMC; Mebendazole[35] in 1% CMC was included as standard reference. All drugs and extract suspensions were freshly prepared before starting the experiment.

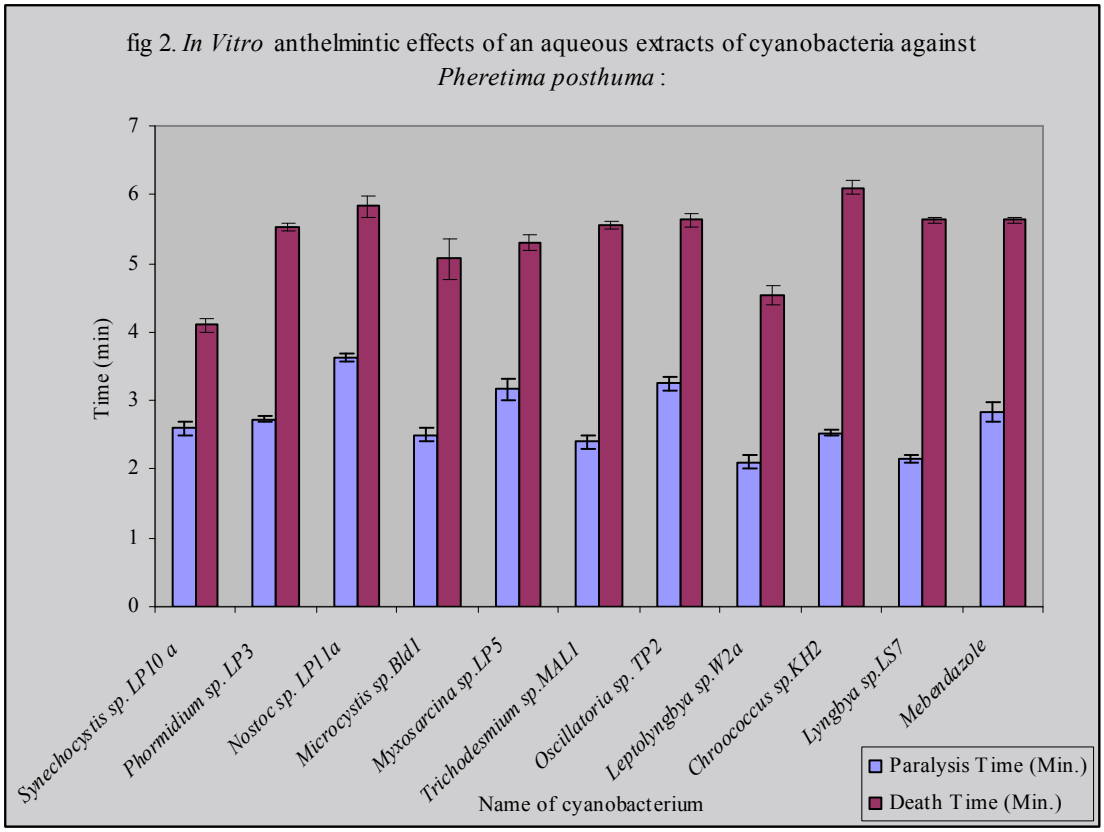
Observations were made for the time taken for paralysis and death of individual worms[36]. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors. The extracts of cultures which have shown positive activities are mentioned here.

Statistical Analysis: Data were expressed as mean of the triplicate values ± Standard deviation, statistically significant at $P<0.05$.

Results and Discussion

Fig 1. *In Vitro* anthelmintic effects of methanolic extracts of cyanobacteria against *Pheretima posthuma* :





Methanolic extracts of some cyanobacterial species viz. *Synechocystis* sp. LP10 a, *Nostoc* sp. LP11a, *Myxosarcina* sp.LP5, *Oscillatoria* sp. TP2 and *Lyngbya* sp.LS7 have shown time for paralysis more closer to the standard whereas others required more time. Slight variations have occurred in death time, in that most of the species have shown significant death responses as compared with that of standard. Whereas strains viz. *Phormidium* sp. LP3 and *Chroococcus sp.* KH2 took comparatively more time.

Moreover, the aqueous extracts of almost all species have shown significant paralytic effects except few strains viz. *Nostoc* sp. LP11a, *Myxosarcina* sp.LP5 and *Oscillatoria* sp. TP2 which took little more time as compared with the standard. Most of the aqueous extracts were taken death time within a significant range except slight deviations in few species viz. *Synechocystis* sp. LP10a, *Leptolyngbya* sp.W_2a and *Chroococcus sp.*KH2 as shown in figure 2.

Cyanobacteria being considered as a source of bioactive material in an anthelmintic studies for number of reasons; some of which includes; the cultural requirements of these organisms are minimum, they are photosynthetic autotrophs, hence these are naturally cost-effective with respect to cultivation and production purposes. Moreover, through literature survey it has been observed that, cyanobacteria have been shown to produce a wide variety of bioactive pharmaceutical compounds[37]. In spite of the studies carried out so far, many cyanobacterial compounds are still largely unexplored, they giving a rich opportunity for discovery of new bioactive compounds[38].

The observations from cyanobacterial methanolic and aqueous extracts have shown that some species interacts and interfere with the *P. posthuma* system immediately whereas others require comparatively some more time, results into either early paralysis or death. The effectiveness of the drug as an anthelmintic response should be as early as possible; this is because its immediate fate in its digestive tract will begin and the transient metabolites may not be as potent as the parent one Though the time for paralysis was less in certain species but the death time was more or vice versa. These differences might be due to some factors influencing interactions in between extract component and the process in which it interferes! In spite of slight variations in the time for paralysis and death in some cyanobacterial species, but these are statistically within the significant range.

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