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Contents

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Research Article

Fungal endophytes associated with the ethnomedicinal plant *Meyna spinosa* Roxb.

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ABSTRACT

Endophytes are a group of microorganism that grow within the tissues of higher plants and colonize them without causing any noticeable injury to the host. Both bacteria and fungi are considered as endophytes. As endophytes represent a potential hub of novel bioactive compounds such as antibiotics, anticancer and other biological control agents, scientific investigations to explore the diversity of endophytic microbes seemed to be significant. *Meyna spinosa Roxb*. belongs to the family Rubiaceae (local name: *Kutkura*) is a medium sized tree with potent medicinal properties. Altogether seven endophytic fungal genera were isolated from the roots, stems and leaves of the target species and thus, maximum fungal species were isolated from various parts of this target plant. More fungus was reported in the stems as compared to the leaves and roots. Fungal identification was based on the colony morphology and examination of spores/conidia or fruiting bodies using stereo and light compound microscopes. *Absidia* sp., *Aspergillus* sp., *Cladosporium* sp., *Cunninghamella* sp., *Fusarium* sp., *Nigrospora* sp., *Paecilomyles* sp., *Penicillium* sp., *Rhizopus* sp., etc. represents among the identified endophytic genera in the present investigation. A large number of sterile mycelia were also observed in association with the target species. Studies on enumeration of endophytic fungal diversity of *Meyna spinosa* and assessing their bioactive potential are important to explore this beneficial microbiota to promote human welfare.

Keywords: Bioactive compounds; Endophytes; Fungi; *Kutkura*; *Meyna spinosa*.

1. INTRODUCTION

Endophytes are ubiquitous endo-symbiontic microorganisms, survives often a fungus or bacterium that exists in healthy living cells of all the plant species studied so far and are known for their beneficial activities to the host. The nature of relationship established by the endophytes with the host plants, however, varies from symbiotic to pathogenic [1]. They may be transmitted either vertically (offspring) or horizontally [2]. Vertically transmitted fungal endophytes are normally considered as sterile and transmit *via* fungal hyphae penetrating the host's seed. Since their reproductive fitness is intimately tied to that of their host plant, these fungi are often considered as mutualistic. Conversely, horizontally transmitted fungal endophytes are fertile, and reproduce through asexual or sexual spores that can be spread by wind or insect vectors. Some endophytic fungi are actually latent pathogens or saprophytes that only become active and reproduce when the host plants are in stress [3].

Endophytes assist the host plants through preventing the colonization from pathogenic organisms into them. Endophytic fungi are gradually more recognized as the potential sources of novel natural products for exploitation in medicine, agriculture and industry with more bioactive natural products isolated from the microorganisms. Therefore, the use of endophytic fungi opens up novel avenues for their biotechnological exploitation, which gradually lead for the isolation and cultivation of these organisms [4].

Meyna spinosa Roxb. belonging to Rubiaceae family, is a shrub with straight, sharp spines growing in hot and humid climate condition. It is locally known as "*kutkura*" (Plate I,A) plant. The whorled green leaves of the plant are arranged in decussately opposite manner. Its flowering season starts in late spring and continues towards early summer. Fruits are berry (Plate 1C). It is distributed in India, Bangladesh, and Nepal and also found in the plain lands of Java and Myanmar [5]. It is very useful medicinal plant. The ripe fruits of target plant are rubbed on cracked heels for quick healing and are also used to cure stomach cancer [6]. Dried fruits are eaten and are also said to be useful in piles. Seed paste is helpful to cure pimples and is also in use in case of abortion [7]. The plant is also reported for its traditional use in the treatment of skin disorders, abortion, ear paining, pneumonia, and *anduria* diseases.

It is believed that medicinal plants and their endophytic flora produce similar pharmaceutical products. The use of endophytic fungus for the production of pharmacologically active metabolites has been on mount [8]. Endophytes are mostly unexplored group of microorganisms, but a few studies report them as a huge source of medicinal compounds. The present investigation was conducted to determine the diversity of endophytic mycoflora of *Meyna spinosa* Roxb. a threatened and ethno medicinally important plant of North East India.

2. MATERIALS AND METHODS

2.1. Sample collection

Healthy leaf, stem and root samples of *Meyna spinosa* were collected from two different areas of Jorhat district, Assam, India. Samples were collected in pre-sterilized polythene bags after removal of excess moisture. The leaf samples were stored at 4°C for further microbial analysis.

2.2. Isolation of endophytic fungi

Asymptomatic healthy plant materials were thoroughly washed in tap water, and surface sterilized [9]. The selected leaf, stem and root segments were immersed in 95% ethanol for 30s, 4% sodium hypochlorite solution for 15s and 95% ethanol for 30s followed by rinsing with sterile distilled water (SDW) three times for 10s and allowed to dry under sterile conditions. After drying, each segment was cut into approximately 0.5 cm squares and placed on petriplates containing potato dextrose agar medium (PDA) supplemented with streptomycin (100 mg/L) to suppress bacterial growth. Petriplates were properly sealed and incubated at 30°C in a light chamber for up to one week. They were monitored every day for growth of endophytic fungal colonies. Fungi growing out from the samples were subsequently transferred onto fresh PDA plates. Sub culturing was continued several times in order to isolate pure colonies.

2.3. Fungus identification

The morphological characteristics of the fungal isolates were examined [10]. Taxonomic identification keys such as colony diameter, texture, color and the dimensions and morphology of hyphae and conidia were recorded using standard protocols [11].

2.4. Determination of colonizing frequency (CF%) of fungal isolates:

The colonizing frequency (CF%) of a single endophytic fungal species in the leaf, stem and root segments was calculated by using the following formula [12].

$$CF\% = \frac{\text{Number of segments colonized by an endophytic fungal species}}{\text{Total number of segments}} \times 100$$

3. RESULTS AND DISCUSSION

In the present investigation, a total number of seven endophytic fungal genera were isolated from the roots, stems and leaves of the target plant species (Fig 1). The highest species richness and colonization frequency was observed in stems as compared to that of leaves and roots. The number of isolated fungal species varied with respect to the stems, leaves and roots. Similar studies on isolation of endophytes has been reported earlier [13-15].

A total of one hundred and thirty two endophytes from different healthy parts of the plants such as leaves, stem, fruits and roots of the four ethnomedicinal plants i.e. *Digitalis purpurea, Digitalis lanata, Plantago ovate* and *Dioscorea bulbifera* [13] were isolated. Altogether 150 fungal and 71 actinomycete endophytes from the internal tissues of woody branches, shoots and leaves of different plants of *Taxus baccata* and *Taxus brevifolia* have been reported [14]. Further, 418 endophyte morpho-species from 83 healthy leaves of *Histeria concinna* and *Ouratea lucens* in a lowland tropical forest of central panama were reported and was proposed that tropical endophytes themselves could be hyperdiverse with host preference and spatial heterogeneity [15].

Table 1. Endophytes isolated from the root stem and leaves of *Meyna spinosa*.

Sl. No.	Isolated endophytes	Root	Stem	Leaves
1	*Absidia* sp.	-	+	-
2	*Aspergillus* sp.	+	-	-
3	*Cladosporium* sp.	-	-	+
4	*Cunninghamella* sp.	-	+	-
5	*Fusarium* sp.	+	+	-
6	*Nigrospora* sp.	-	+	+
7	*Paecilomyces* sp.	-	+	-
8	*Penicillium chrysogenum*	-	-	+
9	*Rhizopus* sp.	+	+	-

+ Present, - absent

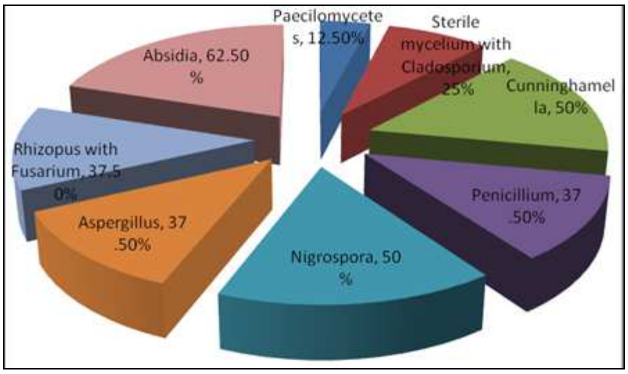

Figure 1. Pie chart showing the colonizing frequency of isolated fungi.

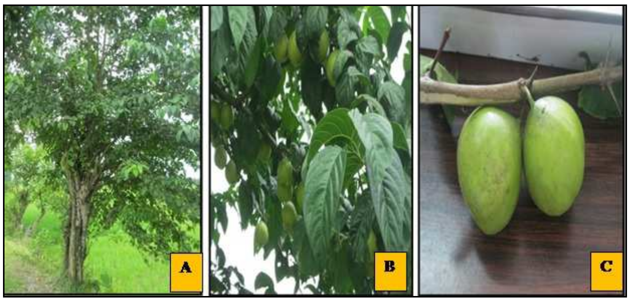

Plate I. A. *Meyna spinosa* habit, B. A fruiting branch, C. Fruits.

The fungal endophytes isolated from the stems were *Paecilomyces* sp., *Cunninghamella sp.*, *Nigrospora* sp., *Absidia* sp., *Rhizopus* sp. with *Fusarium sp.* while *Cladosporium sp., Penicillium chrysogenum, Nigrospora sp.* were isolated from leaves. *Aspergillus* sp., and *Rhizopus sp.* with *Fusarium sp.* were isolated from the roots of the said plant (Table 1). The fungus *Absidia* sp. was found to be dominant endophyte with maximum colonizing frequency (62.50%) followed by *Cunninghamella* sp. (50%), *Nigrospora* sp. (50%), *Aspergillus* and *Cladosporium* sp. (25%), and *Paecilomycetes* sp. (12.50%) respectively. A large number of sterile mycelia were also observed in all the three parts of the target species.

It was observed that the isolated fungi were mostly mitosporic fungi belonging to Hyphomycetes, Coelomycetes and Ascomycetes, while sterile forms were also present as endophytes in the said species. Basidiomycetes and oomycetes were not encountered as these fungi rarely occur as endophytes [9]. The presence of sterile forms as endophytes continues to frustrate mycologists because of their uncertain taxonomy.

However, it is possible to distinguish them from each other by taking into account their culture characteristics. In the present investigation, we have also observed sterile forms as endophytes that were distinguished based on the culture characteristics in media. Sterile forms have been recorded in other angiosperm hosts also [16]. In some cases molecular sequence data from the nuclear ribosomal internal transcribed spacer region have been used to identify sterile cultures [17]. Some hyphomycete genera such as *Curvularia* is common phylloplane fungi, but, are known to occur as endophytes (even in the present study, this fungus occurred as endophyte). However, they are capable of pene-trating the superficial layers of the leaf, when they do so; they survive the rigorous surface sterilization procedures used for isolating endophytes and grow out as colonies in plates [18]. Several workers have reported the occurrence of phylloplane fungi as endophytes from diverse groups of plants. It was suggested that phylloplane fungi might resort to an endophytic mode of life to overcome adverse environmental conditions [19].

Some valuable organic substances are produced by these endophytes that are novel sources of bioactive compounds to solve not only human health but plant and animal health problems also. *Aspergillus* sp., *Cladosporium* sp., along with various beneficial fungi such as *Paecilomyces* sp., *Cunninghamella* sp., and *Penicillium chrysogenum* were isolated here. *Paecilomyces* sp. is a nematophagous fungus and shows a big promise as biological control. *P. chrysogenum*, is source of several β-lactam antibiotics [20]. *Cunninghamella elegans* is able to degrade xenobiotics and used as a microbial model of mammalian drug metabolism. *Cunninghamella sp*. has also been used in environmental biotechnology for the treatment of textile waste waters for instance those discoloured by azo dyes or malachite green.

CONCLUSION

The study aids adequate knowledge towards fungal endophytes in regards to their occurrence in medicinal plants. It is apparent that these fungi might have significant impacts on the survival and fitness of plants in all terrestrial ecosystems and therefore likely to play crucial role in plant biogeography, evolution and community structure. Isolation of endophytic fungi from medicinal and other plants may result in methods to produce biologically active agents for biological utilization on a large commercial scale as they are easily cultured in laboratory instead of harvesting plants and affecting the environmental biodiversity.

AUTHOR'S CONTRIBUTION

LHB and GB contributed in field work, collection and laboratory isolation of the fungal endophytes, the manuscript preparation and literature review associated with this research article. The final manuscript has been read, carefully scrutinized and compiled by both the authors and approved the same. VP helped in identification of the isolated endophytes. PNB assisted in final manuscript modification and scrutinization.

TRANSPARENCY DECLARATION

The authors declare no conflict of interest.

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Research Article

Notes on two species of *Zygnema* C. Ag. with water analysis from Hooghly in West Bengal, India

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ABSTRACT

This paper includes a general study of two species of the genus *Zygnema* C. Ag., from the viewpoint of taxonomy and ecology. In this work, two interesting species *viz*. *Zygnema cruciatum* (Vauch.) C. Ag. and *Zygnema czurdae* Randh. of the class Chlorophyceae (green algae) have been taxonomically described. Simultaneously, physico-chemical analysis of selected parameters (temperature, pH, dissolved oxygen, biological oxygen demand, nutrients, turbidity and total alkalinity) of water was done to assess water quality and its impact on their occurrence and growth in water bodies. From this study, alkaline pH, higher value of DO, turbidity and TA as $CaCO_3$ were recorded. High turbidity value of water indicated presence of contaminations within ponds whereas values of nitrate-nitrogen and phosphate were found sufficient for growth of those aquatic green algae. Both the algal species were collected from stagnant water body (pond) located at two different sites in Hooghly, West Bengal, India. Under natural condition, they were mixed in community i.e. grown in association with other green filamentous algal genera. Limnological study also exhibited that their phenology was dependent on variations of physico-chemical characteristics of water. All these species constitute new taxonomic reports from Hooghly of West Bengal, India.

Keywords: *Zygnema*; Taxonomy; Water analysis; West Bengal; India.

1. INTRODUCTION

Algae are very important component of aquatic environment like other aquatic flora and fauna. Macroscopic algal communities including zygnematales play a key role as primary producer and generate sufficient biomass in aquatic ecosystems. In addition to these, they help in oxygen enrichment in water and can remove or tolerate certain toxic matters like lead (Pb), copper (Cu), cadmium (Cd), inorganic arsenic (arsenate or arsenite) etc. through adsorption and biotransformation in wet lands. It has been established that several biotic and abiotic factors or their interactions can influence their growth in water bodies. Thus, presence of algal community is very important in aquatic ecosystems for improvement of water quality, phytoremediation and to sustaining the ecology of water bodies. Filamentous conjugating algae are most well known and important members of class Chlorophyceae and grow every type of aquatic ecosystems as free floating or attached forms. They are frequent in stagnant water of roadside ditches, paddy fields, ponds and lakes in tropical climatic conditions where they can form free-floating mats or intermingle with other algae [1]. They exhibit higher photosynthesis, growth and produce large biomass [2].

Zygnema C. Ag. (Zygnematales, Chlorophyceae) is a very common, widespread, green, filamentous and unbranched conjugate algal genus [3]. It shows the following diagnostic features:

i) thallus is filamentous, unbranched not very long, bright green in vegetative condition, enveloped by a soft mucilaginous sheath,

ii) they may be attached to a substrate by means of rhizoid,

iii) vegetative cells cylindrical, length is equal to or greater than width; uninucleate with 2 characteristic axial stellate chloroplasts, each containing a single large central pyrenoid,

iv) cell wall of vegetative cells two-layered, inner is cellulosic whereas outer one mucilaginous, thinner and end walls plane,

v) vegetative multiplication occurs by means of breaking of filaments,

vi) asexual reproduction takes place by aplanospores and akinetes,

vii) sexual reproduction occurs by means of conjugation process,

viii) conjugation is scalariform or lateral,

ix) zygospore (sexual spore) formed in one of the gametangia or conjugation canal,

x) zygospore spherical or compressed-globose to ovate or ellipsoid, thick walled and brown or blue; mature zygospore wall is usually three layered; outer and middle layers generally ornamented; ornamentation is considered as crucial for identification of species,

xi) most of the species of the genus are homothallic and life cycle is haplobiontic type. In natural conditions, it grows in the same type of habitats where *Spirogyra* Link is grown and often found intermingled with it [4].

Pond is a very common and essential aquatic habitat that harboring different types of algal flora in different seasons such as winter, spring, summer and autumn throughout the year. The availability of water as well as substratum, temperature, sun light and nutrients may result the flourish of zygnematalean algae in this water body. In the present work, several water parameters such as temperature, pH, DO, BOD, nitrate-nitrogen, phosphate, turbidity and TA that contribute vital role for their occurrence and growth in the water body have been determined/measured and analyzed.

The genus *Zygnema* C. Ag. includes a large number of species and till now 204 species have been currently accepted taxonomically from all over the world [5] but the taxonomical works on this taxon is still scanty in India. Some important pioneering works in the field of taxonomy, systematic survey and distribution of this genus from different regions of India may be cited [6-9]. It was Randhawa [10] who made a commendable or noteworthy work on the members of Zygnematales through his publication of the monograph 'Zygnemaceae' from India. Probably, he made first contribution to our knowledge of this genus by reporting three new species from northern region of India [11].

Algae possess some bioactive compounds/ secondary metabolites which are responsible for antibacterial/antimicrobial, antioxidant potentiality [12-18] as well as they have the ability to produce silver nanoparticles (AgNPs) by reduction of silver nitrate [19]. Methanolic extract of *Zygnema* revealed the presence of saturated and unsaturated fatty acids (FAs) through GC-MS (Gas chromatography-mass spectrometry method) and beta-sitosterol by ^{1}H-NMR spectrum. In *Z. czurdae* one saturated, three monounsaturated and one triunsaturated FAs were detected earlier [20]. β-sitosterol is a phytosterol ester which is commonly used for the treatment of heart disease, to decrease high cholesterol level, preventing colon cancer and boosting up the immune system [21-22].

Thus, there has been very little validated work on taxonomic study of this genus of prior this report from India. Due to having some applied applications and found a lacuna for documentation of those species in West Bengal henceforth, this work was undertaken.

2. MATERIALS AND METHODS

The algal samples were collected in plastic packets from two ponds located at Bansberia (22°.95'N; 88°.40'E) and Behula (23°.18'N; 88°.42'E) of Hooghly district, West Bengal in India. They were examined using the light microscope Olympus (Model-CH20i) and the voucher specimens were deposited in the Dept. Herbarium of Raja Peary Mohan College, Uttarpara, West Bengal.

2.1. Identification of *Zygnema* species

Two species were identified following the standard scientific publications and monographs [8, 10-11, 23-26]. Each currently accepted name has been provided with its author's name.

2.2. Quantitative estimation of water quality

Selected limnological parameters were determined using standard methods described in APHA [27] and earlier by authors [28-36].

The temperature (°C) of the water sample was recorded using Zeal's (UK) Mercury thermometer on the spot.

pH of water sample was measured at the spot immediately after collection with the help of portable digital pH meter (Merck, Germany, Model No. 320).

DO (dissolved oxygen) was analyzed using Rideal-Stewart modification [37] of Winkler method following the procedure laid down in APHA [27].

BOD (biological oxygen demand) was determined by the 5-day BOD test through titrimetric method of APHA [27]. Before initiating the BOD_5 test, air tight special BOD glass bottles were kept in incubator at 10^0C temperature for 5 days.

In this experiment, quantitative analysis of NO_3-N (Nitrate-nitrogen) was performed by the Brucine method. For this, first acidification of water sample was done with 1(N) HCl to prevent interference from hydroxide or carbonate concentrations. Following acidification, different reagents like brucine-sulfanilic acid soln., sulfuric acid (H_2SO_4) soln., sodium chloride soln. (300 g/l) and nitrate ion standard soln. (1 mg NO_3-N/l) were mixed up with 10 ml water samples as described in the Brucine procedure. Then UV-VIS spectrophotometric (CECIL CE 7200) readings of solutions were taken separately at OD_{220} and OD_{275} absorbance. The OD_{275} value was multiplied by 2 and subtracted from OD_{220} value. After subtraction, actual OD values were calculated and plotted in a standard NO_3-N curve. The calibration curve was prepared by plotting the absorbance of standards run by the above procedure against concentration in mg NO_3-N/l.

PO_4^{3-} (Phosphate) was analyzed by standard method described in APHA [27]. The filtered water sample was treated following with stannous chloride ($SnCl_2$) method. Here 0.4 ml NH_4- molybdate and 2-3 drops of $SnCl_2$ reagents were mixed up with 10 ml water sample and then molybdenum-blue complex was formed. After 10 minute but before 12 minute UV-VIS spectrophotometric reading at OD_{690} absorbance was taken. After measuring the absorbance of the solution, a standard PO_4^{3-}curve was prepared by plotting absorbance against concentration in mg PO_4^{3-}/l.

As pH of water sample was found below 8.3, the TA (Total alkalinity) was determined by titrating a portion of sample (50 ml) with standardized sulfuric acid to pH 4.5 end point procedure using 5 drops of bromocresol green-methyl red as indicator solution.

The turbidity of collected water sample was measured by taking direct reading from Nephelometer (model CL-52D) after calibration of turbidity meter by 4000 NTU standard solution (mixture of 5 ml of hydrazine sulphate and 5 ml of hexamethylene tetramine with 90 ml distilled water).

3. RESULTS AND DISCUSSION

In this paper, two species of the genus had been described taking only the morphological features of vegetative and reproductive cells.

Key to the species:

1(a) Vegetative cells 25.0-28.0 μm broad; zygospores brown and formed in one of the gametangia - *Zygnema cruciatum*

1(b) Vegetative cells 20.0-23.0 μm broad; zygospores bluish, formed in conjugation canal and endospore slightly sinuous - *Z. czurdae*

Order: Conjugales; Family: Zygnemaceae

Genus: *Zygnema* C. Ag.

3.1. *Zygnema cruciatum*

Zygnema cruciatum (Vauch.) C. Ag. in Syn. Alg. Scand. 102, 1817; [24] Zygnemales 246, fig. 291-292, 1941; [10] Zygnemaceae 243, fig. 197, 1959; [25] Zygnemacées Africanines 64, pl. 18, fig. b, 1965; [8] Alg. Fl. Bihar 23, 2004 (Figure 1. A-C). *Conjugate cruciata* Vaucher.

Taxonomical description:

Vegetative features: Filamentous, unbranched; vegetative cells cylindrical or inflated, without base and apex differentiation, vegetative cells 25.0-28.0 μm broad and 65.0-85.0 μm long; septa plane; chloroplasts two and stellate/star shaped; pyrenoids two; nucleus single, centrally located.

Reproductive features: Conjugation both scalariform and lateral; zygospores mostly spherical to cylindric-ovoid, 27.0-30.0 μm broad and 27.0-32.0 μm long; formed in one of the gametangia; receptive or female gametangia cylindric or enlarged; median spore wall (mesospore) scrobiculate and brown; pits about 1.5-2.0 μm in diameter.

Remarks: It is very similar with *Zygnema normani* Taft in terms of breadth of vegetative cells, zygospores formation in one of the gametangia (not within conjugation canal) and their sculptures (scrobiculate or pitted) but differs from colour and width of zygospores and pits diameter. In the present specimen, breadth of vegetative cells and size of zygospores are slight smaller than the original specimen. It should be mentioned that this is the second time report from West Bengal, India.

Distribution note:

India: Punjab, Maharashtra, Bihar, West Bengal [38].

Outside of India: Europe: Britain [39-40], Latvia [41], Romania [42], Slovenia [43], Spain [44-45]. Africa: Algeria, Egypt, Morocco, Tunisia [25]. North America: USA [46]. South America: Chile [47]. Asia: China [24], Manchuria [24], Japan [48], Libya [49], Iraq [50], Russia [51]. Australia and New Zealand: New South Wales, Tasmania, Victoria [52], Queensland [52, 54], New Zealand [53] as mentioned [5].

Habitat: Pond water at Bansberia (site-1), Hooghly in West Bengal, India.

Collection No: NH ZYG 4; dated: 06.12.2015.

Phenology: November-February (winter).

Ecological note: Free floating in association with other filamentous alga like *Spirogyra* Link; filaments brown-green, unbranched, thinner and less slippery in touch.

Significance: The species is a primary producer and serves as an important food source for some fishes.

3.2. *Zygnema czurdae*

Zygnema czurdae Randh. in Proc. Indian Acad. Sci. 4(3): 239, pl. 11, figs. 1-7, 1936; 8(3): 137-138, fig. 22, 1938; [23] in Proc. Nat. Acad. Sci. India Biol. Sci. 201-204, figs. 1-9a, 1959; [26] in Biblioth. Phycol. 66: 254, 1984 (Figure 1. D-E).

Taxonomical description:

Vegetative features: Thallus filamentous; filaments bright green, unbranched and free floating; cells are cylindrical; vegetative cells 20.0-23.0 μm broad; nearly four times as long; two star shaped, more or less rounded chloroplasts with two conspicuous pyrenoids in each vegetative cell.

Reproductive features: Conjugation mostly lateral or scalariform; in scalariform conjugation, zygospores form in the conjugation canal, filling whole of the conjugation canal area and distended; zygospore wall three layered, exospore and mesospore smooth while endospore slightly sinuous; zygospores bluish, 30.0- 36.0 μm broad; oval in shape (in early stage) but later become rounded.

Remarks: The present species resembles with *Z. indicum* Misra in its compressed zygospore while it differs from *Z. Carterae* Czurda in the size of vegetative cells and zygospores. This is probably first report from this state in India.

Habitat: Pond at Behula (site-2), Hooghly in West Bengal, India.

Collection No: NH ZYG 5; dated: 04.01.2016.

Phenology: December to 1st week of March [winter-beginning of summer].

Ecological note: Free floating with other filamentous algal assemblages including *Mougeotia* C. Ag.; filaments unbranched, light greenish and entangled in the pond.

Distribution note:

India: Punjab, Uttar Pradesh, Andhra Pradesh and Jharkhand [38].

Outside of India: Europe: Turkey (Europe) [55]. Asia: Pakistan [56], Iraq [50] as stated [5].

Significance: It acts as a primary producer in aquatic ecosystem.

A key goal of taxonomic survey is the exploration and documentation of species with their geographical distributions [57-58]. The order Zygnematales consists of the conjugating green algae and this order is the largest and most diverse among the Chlorophyceae (green algae). Their members are most frequent in freshwater streams, ponds, lakes and reservoirs. It has been observed that tropical climatic condition that is prevailing in India is favorable for the growth of green filamentous algae [59] as well as blue-green algae in various aquatic habitats. Species of *Zygnema* were found as free floating form associated with other filamentous algae like *Spirogyra* Link and *Mougeotia* C. Ag. The phenology including dates of collections revealed that these two species appeared in the beginning of winter (late November to December) and then gradually formed sexual reproductive organs (fruiting stages) and live till at the end of winter month (February) or beginning of summer (March). After that, they gradually declined in the water bodies perhaps, surface water was become warmer more than 30°C during summer which was not suitable for their growth.

In taxonomic work, measurements of breadth and length of vegetative cells and zygospores, conjugation type as well as shape, position and colour of zygospores formed in the receptive gametangium have been used as morphological characteristic features for distinguishing the species. In *Zygnema cruciatum* (Vauch.) C. Ag., vegetative cells 25.0-28.0 μm broad, zygospore brown, 27.0-30.0 μm broad, pits 1.5-2.0 μm broad (Figure 1. A-C). Conjugation was observed either scalariform or lateral. When it is scalariform, the conjugation canals are formed by both of the gametangia and the longitudinal wall of the receptive gametangium getting slight inflated towards right conjugating side near the cross wall. Similar phenomenon was also observed [60].

Zygnema czurdae Randh. was first reported from a fresh water spring at Tahli Sahib in Hoshiarpur district, Punjab, India [11]. After that it was documented from other three states of India as mentioned in distribution note excluding the state of West Bengal. Therefore, it is the first time report for this state. In *Z. czurdae* Randh., vegetative cells were 20.0-23.0 μm broad and conjugation was scalariform. Zygospore smooth and oval or rounded and endospore was found slightly sinuous (Figure 1. D-E).

Water plays a pivotal role in the distribution of algae [61] in wet lands. Study of the physico-chemical properties of water gives valuable information/data of the aquatic environments where algae are grown or live. Specially, fluctuations of temperature and nutrient condition influence the algal abundance, biomass etc. in the aquatic ecosystems [62-63]. The results of assessment of environmental variables/water parameters of the two algal collection sites were presented in Table 1.

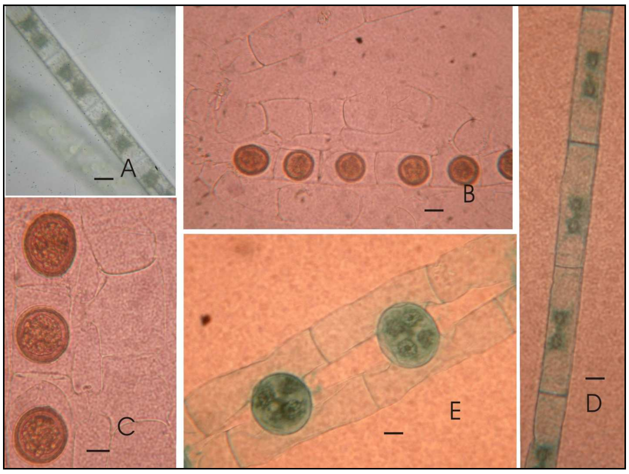

Figure 1. A-C. *Zygnema cruciatum* (Vauch.) C. Ag., D-E. *Zygnema czurdae* Randh. bar = 25 μm.

Table 1. Measured physico-chemical parameters of water at sampling sites.

Sl. No.	Physico-chemical parameters	Bansberia (site 1)	Behula (site 2)
1	Temperature (ºC)	18.5ºC±0.25	18.0ºC±0.25
2	pH	7.7±0.05	7.9±0.05
3	DO (mg/l)	7.0±0.12	7.4±0.12
4	BOD (mg/l)	3.8±0.10	3.2±0.11
5	NO_3-N (mg/l)	0.15±0.07	0.20±0.05
6	PO_4^{3-}(mg/l)	0.28±0.08	0.32±0.10
7	Turbidity (NTU)	22±0.57	26±0.1.1
8	TA as $CaCO_3$ (mg/l)	252±2.3	290±2.8

All the parameters are in triplicate values with mean value ± standard error mean; DO = Dissolved oxygen, BOD = Biological oxygen demand, NO_3-N = Nitrate-nitrogen, PO_4^{3-} = Phosphate, TA = Total alkalinity.

Temperature is an important parameter which influences chemical and biological reactions in water and it affects sustenance of living biota in ecosystems. Surface water temperature of study sites was measured from 18.0-18.5°C. Lower temperature of water was reported during winter which could be due to the influence of cooler climatic condition or low atmospheric temperature. pH is measured by the negative logarithm of hydrogen ions concentration. The pH of natural water is greatly influenced by the concentration of CO_2 [64]. pH of water sample was recorded alkaline (7.7-7.9) which reflected increased rate of photosynthetic activity in the water bodies. This was also in accordance with earlier reports [65-66] and showed direct relationship between pH and temperature as well as established important role of pH for the occurrence of those green algal species in water body. Dissolved oxygen (DO) affects the growth, distribution and physiology of aquatic organisms. DO concentration more than 5.0 mg/l favours suitable growth of flora and fauna in water [67]. In this study, it was observed from 7.0-7.4 mg/l in two ponds which was slight higher like the previous observations [68-69]. Actually, the amount of dissolved oxygen in water is dependent on some factors like water temperature, photosynthetic activity, rate of respiration and organic loads that exist in water bodies. The higher values of DO in the studied aquatic bodies might be due to higher solubility of oxygen at relatively lower temperature in winter. BOD is the measurement of total dissolved oxygen consumed by microorganisms for biodegradation of organic matters. Here, BOD values were observed lower in the site-2 and higher in the site-1. Most importantly, DO and BOD values showed negative interactions with each other which were also evident while studying pond water at Tribeni, in West Bengal, India [70]. Thus, both ponds exhibited high organic load.

NO_3-N and PO_4^{3-} are two essential nutrients that stimulate growth of algae and directly or indirectly increase the productivity of aquatic ecosystems. Amount of NO_3-N and PO_4^{3-} was found low similar to the earlier result [71]. Low concentration of nutrients in ponds might be attributed to the utilization by floating hydrophytes or other autotrophs like algae. While, turbidity and TA values were noted comparatively greater in site 2 as compare to site 1. Total alkalinity is a measurement of the total concentration of bases in water including carbonates, bicarbonates, hydroxides, phosphates, dissolved calcium, magnesium, and other compounds and it is also responsible for changing of pH of water [72]. Probably, photosynthesis as well as denitrification helped for increase the overall alkalinity values in those two sites. The total alkalinity was found 252.0 and 290.0 mg/l in two ponds. These values may be considered high like the result of another work by author [73]. The limnological study indicated that both the ponds were still productive as TA value was observed quite high (over 90.0 mg/l). In addition to that the result of TA also suggested presence of high amount of dissolved carbon dioxide that perhaps permitted/allowed those algae to rise in the ponds. Turbidity is the expression of status of water in respect of suspended particles and it is an effective indicator of water quality [74]. Various factors like clay, silt, fine organic and inorganic matter, algae and other microscopic organisms are responsible for turbidity of water sample. It is well known to us that water with high turbidity is cloudy, while water with low turbidity is clear and it become visibly turbid at levels above 5 NTU. In this investigation, turbidity varied between 22.0-26.0 NTU in the two algal collection sites. The maximum permissible limit of turbidity as prescribed by BIS [75] is 10 NTU. Here, it was noticed to cross the permissible limit. The input and presence of high turbidity had been attributed to greater amounts of suspended matters including phytoplankton in the studied sites. Wetzel [76] stated that dissolved oxygen affects solubility and availability of the nutrients which greatly influences the productivity of the ecosystem. Similar finding was observed in this study. Thus, water analysis suggested that the two sites were loaded with suspended particles and both the ponds were productive in terms of nutrients and total alkalinity values.

5. CONCLUSION

This study basically provided the taxonomic characteristics of two species of *Zygnema* and fundamental ecological information of water bodies (ponds). It also indicated that winter season, lower temperature (18-18.5°C), alkaline water with quite high turbidity and TA values as well as low nutrient contents of water facilitated their abundance, growth and distribution in ponds. A tendency to grow in turbid water of those two species had been also noticed. Therefore, characterizations of physico-chemical parameters in respect of algal floral assemblages have a great importance in the study of algal ecology as it reflects a close relationship between these two variables.

TRANSPARENCY DECLARATION

The author declares no conflict of interest.

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