Decoding the influence of limnological parameters favouring cohabitation of microalgae and greater duckweeds in Lentic Ecosystems of Malda District: An attempt with Artificial Neural Network

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Abstract
Sharing of ecological resources among the various species in any ecosystem is a characteristic feature in any living association. In aquatic ecosystem, a particular niche is shared by a number of species and cohabitation is one of the normal adaptive strategies followed by aquatic flora and fauna. In the study, we observed stable spatio-temporal association between greater duckweeds and microalgae in sufficiently screened waterbodies in Malda district of West Bengal.

Collection sites represent various biochemical parameters included in the list of independent variables like pH, TDS, nitrate and phosphate levels etc. Statistical model like ANN explains the role of independently variable limnological parameters towards predicting most significant association and the threshold sample size.

Keywords: Duckweeds, *Spirodela polyrrhiza* (L.) Schleid., Cohabitation, Artificial Neural Networking (ANN), Collection Site (CS), Cyanobateria.

Introduction

Artificial Neural Networking (ANN) is a mathematical model inspired from biological models and follows processing of information similar to that of human brain. The history of neural networks may be dated back to year 1943. Neural networks show an extraordinary ability to convert unorganized or complicated data into patterns that are beyond the scope of human brains or normal computer programs. ANN works by recognizing patterns and establishes relationship between them based on experience similar to human brains[13].

Conventional computer program is based on Algorithms and uses a pre-selected set of instructions to solve a problem whereas neural networks uses experience to solve a problem. Artificial Neural Network is made up of hundreds of single processing units similar to that of human brains that are interconnected with each other.

Such units are called Nodes or Processing elements (PE) because they are known to process information similar to that of neurons found in human brain[25]. Such PE has one input component, transfer component and one output which basically balances the inputs and outputs with an equation. This could be correlated with neurons of human brains made up of axons cell bodies and dendrites[26]. The arriving signals are called inputs which are then multiplied and combined through transfer signals and then processed for output signals.

In ANN, the variables are introduced independently and maximum data against each variable are introduced in the network. In such cases, the best possible combinations were selected which will guide towards the acceptable results and the other unexpected combination are eliminated[9]. The concept of ANN uses two paradigms of learnings, one with supervised learning where there is a possibility to verify the results and the other unsupervised learnings where there is no possibility to verify the results[23]. Artificial Neural Network is applied to a number of fields of biology and agricultural sciences like relationship between pathogen attack and yield of crops[3], absorption of water and mineral elements from the soil[19], prediction of plant virus in causing a particular disease and its evolutionary relationship[10].

Duckweeds are fast growing aquatic monocotyledons abundant in eutrophic water bodies of tropical countries and are represented by five genera *Lemna, Wolffia, Spirodela* , *Wolfiella* and *Landoltia sp* belonging to the family Lemnaceae under the order Alismatelis[21]. Their plant body consists of 2-4 poorly differentiated fronds which are separated by large vacuoles to maintain buoyancy. Their diploid chromosome numbers vary from 2n=20 to 2n=126[17]. Urbanisation has led to decline of many aquatic plant species whereas duckweeds have survived in these harsh environments and continue to grow if undisturbed by external factors and can double their biomass in 48-72 hours.

Spirodela polyrrhiza (L.) Schleid. commonly known as giant duckweed or greater duckweed is cosmopolitan in eutrophic water bodies of the tropical countries with a fast reproductive rate and short life cycle. Their growth causes shade in the water bodies causing eutrophication and is capable of uptaking ammonia and phosphorus from the surrounding water bodies. Their plant bodies are represented by fronds with numerous roots attached to their dorsal surface. They are reproduced by means of vegetative propagation where the daughter fronds remain attached to the mother fronds with the help of connective stalk. *S.*

polyrrhiza (L.) Schleid is known to produce hibernating structures called turions, which on return of favourable condition develop into new plants.

Turions may be compared with seeds of higher plants with respect to their formation, dormancy, germination and are accelerated by phosphate deficiency in the water bodies[2]. Association between algae and duckweed was reported in *Lemna* species in the water bodies of South America. The fronds of *Lemna minor* L. and *Lemna gibba* are often found to be infested with cyanobacterial colonies[24]. Such associations were also found in the reproductive pockets or in the attached roots of *Lemna*[20].

Previous reports have shown that heterocystous blue green algae like *Gloeotrichia* sp,, *Nostoc* sp. , *Cylindrospermum* sp., *Calothrix* sp. and *Anabaena* sp were found as epiphytes on duckweeds by thriving on organic debris released by them creating a suitable microhabitat.

Malda district is situated in the state of West Bengal which is surrounded by the river Ganga and its tributaries. The area is characterized by numerous low lying, small to medium sized stagnant water bodies locally called "Daha" where duckweeds grow abundantly. However, there were no previous reports on the nature of algal association with fronds of greater duckweeds. This study throws pioneering insight on algae-greater duckweed cohabitation in the Malda district of West Bengal simulating the physical and chemical parameters of such water bodies that support such natural association between two evolutionary distinct plant groups with the help of Artificial Neural Network.

Material and Methods

Collection and Identification of plant materials: For the study of algal association with fronds of *Spirodela polyrrhiza* (L.) Schleid., 15 different collection sites abbreviated as CS were selected (CS I- CSXV) randomly and were visited separately in two different seasons viz. summer and winter of 2021. The duckweed fronds from different collection sites were kept in sterile plastic containers of 100 ml volume. The details of the collection site along with the geographical details of the collection sites are given in table 1. The duckweed specimens collected from 15 different collection sites were identified with the help of Central National Herbarium (CNH), Shibpur, Howrah.

Preparation of the plant samples for SEM: To study the algae-duckweed, first the fronds were surface sterilised using 0.1% mercuric chloride solution for 30 seconds to 1 minute followed by washing in double distilled water. The surface sterilized fronds were dehydrated following the chemical fixation procedure using 2.5% gluteraldehyde for overnight[18].

It was followed by dehydration in different grades of alcohol. (50%, 70%, 80%, 95%). The dehydrated plant materials were then subjected to Scanning electron microscopy (SEM) in University Science Instrumentation Centre (USIC), The University of North Bengal, West Bengal using JEOL (JSM-IT 100). The machine was operated using secondary electron detector of 5.0kV and working distance 10nm, probe current PC-#30 and accelerating voltage 5.0kV.

Table 1
Details of Collection sites (CS) of Duckweeds from Malda District along with their geographical coordinates

S.N.	Collection site	Abbreviated name	Latitude (DD)*	Latitude In DM	Longitude (DD)*	Longitude DM
1	English Bazar	CSI	25.004101	$25^0$14„ (N)	88.136797	$88^0$85„ (E)
2	English Bazar	CSII	25.017052	$25^0$01‚ (N)	88.126833	88^0 07„ (E)
3	Old Malda	CSIII	25.012480	$25^0$04‚ (N)	88.153755	88^0 90„ (E)
4	Old Malda	CSIV	25.065583	$25^0$08‚ (N)	88.143261	$88^0$08„ (E)
5	Mangalbari (old Malda)	CSV	25.025516	$25^0$11 „ (N)	88.146586	$88^0$48„ (E)
6	Kaliachak I	CSVI	24.882428	$24^0$52 „ (N)	88.013306	$88^0$04„ (E)
7	Kaliachak I	CSVII	24.880258	$24^0$54„ (N)	88.028318	$88^0$10„ (E)
8	Kaliachak II (Paglaghat)	CSVIII	24.92355	$24^0$60„ (N)	87.975536	$87^0$58„ (E)
9	Gazole	CSIX	25.211174	$25^0$12„ (N)	88.179049	$88^0$14„ (E)
10	Gazole	CSX	25.200940	$25^0$14„ (N)	88.181436	$88^0$16„ (E)
11	Gazole	CSXI	25.227241	$25^0$16„ (N)	88.191596	$88^0$17„ €
12	Chanchal	CSXII	25.390265	$25^0$23 „ (N)	88.015690	$88^0$05„ €
13	Chanchal	CSXIII	25.398125	$25^0$32„ (N)	88.014451	$88^0$02„ (E)
14	Aiho	CSXIV	24.959821	$24^0$67„ (N)	88.240144	$88^0$14‚ €
15	Pakuahat	CSXV	25.121945	$25^0$66„ (N)	88.363074	$88^0$23„ (E)

Assessment of Limnological Parameters: Five limnological parameters were studied in all the 15 CS namely pH, total dissolved solids (TDS), turbidity, total nitrate and phosphorus in both the seasons. The pH of the 15 water bodies was measured using a portable pH meter (HM Portable pH meter HD 300). Similarly, TDS was measured using portable TDS meter (HM Digital HM4) and turbidity of 15 CS was measured following Nephelometric method determining the amount of light scattering in the waterbodies[15]. The level of nitrate in the water of 15 CS was determined as per Trivedy and Goel[22], using sulphonic acid and phenol water bodies. The phosphate level in the water was measured following the protocol of American Public Health Organisation[1] using sodium molybdate and orthophosphoric acid.

***In vitro* growth of the greater duckweeds and microalgae:** To study the *in vitro* association between algae and greater duckweeds, collected fronds of *Spirodela polyrrhiza* (L.) Schleid (L.) were maintained in our tissue culture laboratory in pH 5.8, relative humidity 100%, photoperiod 16:8, light intensity 22.2 μmol $m^{-2}s^{-1}$ and temperature 28 ± 2^0C in SH basal medium (HiMedia PT059) supplemented with BG11 broth in 2:1 v/v proportion in having diameter 100 mm and depth 17mm. The entire set up was established in three replicates.

Artificial Neural Network Modelling: Statistical analysis was performed using SPSS v 24.0 software (IBM) version. The different limnological parameters such as collection sites, pH, turbidity, TDS, nitrate, phosphate etc. were considered as independent variables and the algal groups like cyanophyceae (blue-green algae) and bacillariophyceae (diatoms) were maintained as the dependent variable to screen out the significantly higher score of influence of the dependent variables.

The total numbers of collection sites were partitioned into four ratios viz-60/40, 70/30, 80/20 and 90/10 as training: testing to feed in the model algorithm since as per the criteria of the ANN. The significance of the fitment of model is to be ascertained on the logic that more than 50 percent of testing sites is required to supply the data as dependent variables to create a multivariate model and the efficacy of the model to be tested on the values of the dependent variables extracted from rest of the testing sites. The particular segregation ratio at which the relative error is least deviated from one another, is to be considered as the data source for obtaining most significantly 'close-fit' ANN model.

Results and Discussion

The details of the 15 Collection sites (CSI-CSXV) with geographical coordinates are given in table 1. The pH of the water bodies of the 15 CS was found to be in the range of 6.9- 8-5 which indicates that slightly acidic to moderate alkaline pH supports the coexistence of duckweed – algae in both summer and winter. Turbidity (in NTU) was recorded in the range of 8-14 in 15 CS which indicates that non-potable waters are preferred by algae and duckweed to coexist. Water bodies which are infested with algal blooms, show turbidity in the range of 8-15 NTU in China[12]. The nitrate and phosphate levels were higher in all the 15 CS which indicate that both were necessary for their coexistence. Aquatic macrophytes like *Lemna* and *Spirodela* are known to accumulate nitrogen and phosphate from their surrounding water bodies[4]. All the limnological parameter seasonwise are given in table 3 and table 4.

Table 2
Association between algae (along with their names) and *Spirodela polyrrhiza* in all the 15 collection spots of Malda district of West Bengal, India. (+ and ++ indicate presence in low frequency and high frequency respectively and – indicates absence of the individual algae in that collection spots)

Collection sites	*Nitzschia sigmoidea* (Nitzsch) Smith	*Anomoeoneis sphaerophora* Pfitzer	*Navicula gottlandica* Grunow	*Gomphonema olivaceum* (Hornemann) Brebisson	*Oscillatoria jenensis* Schmid	*Oscillatoria sancta* Kutzing and Gomont
CSI	--	++	--	++	--	++
CSII	++	+	++	++	++	++
CSIII	--	--	++	---	--	++
CSIV	--	--	++	--	--	++
CSV	--	--	--	--	--	++
CSVI	--	--	--	--	--	++
CSVII	--	--	--	--	--	++
CSVIII	+	++	--	+	++	++
CSIX	++	--	++	--	++	++
CSX	+	--	++	--	++	++
CSXI	-	++	--	++	--	++
CSXII	--	--	++	--	--	++
CSXIII	++	--	--	--	--	++
CSXIV	--	--	--	--	--	++
CSXV	++	--	--	--	--	++

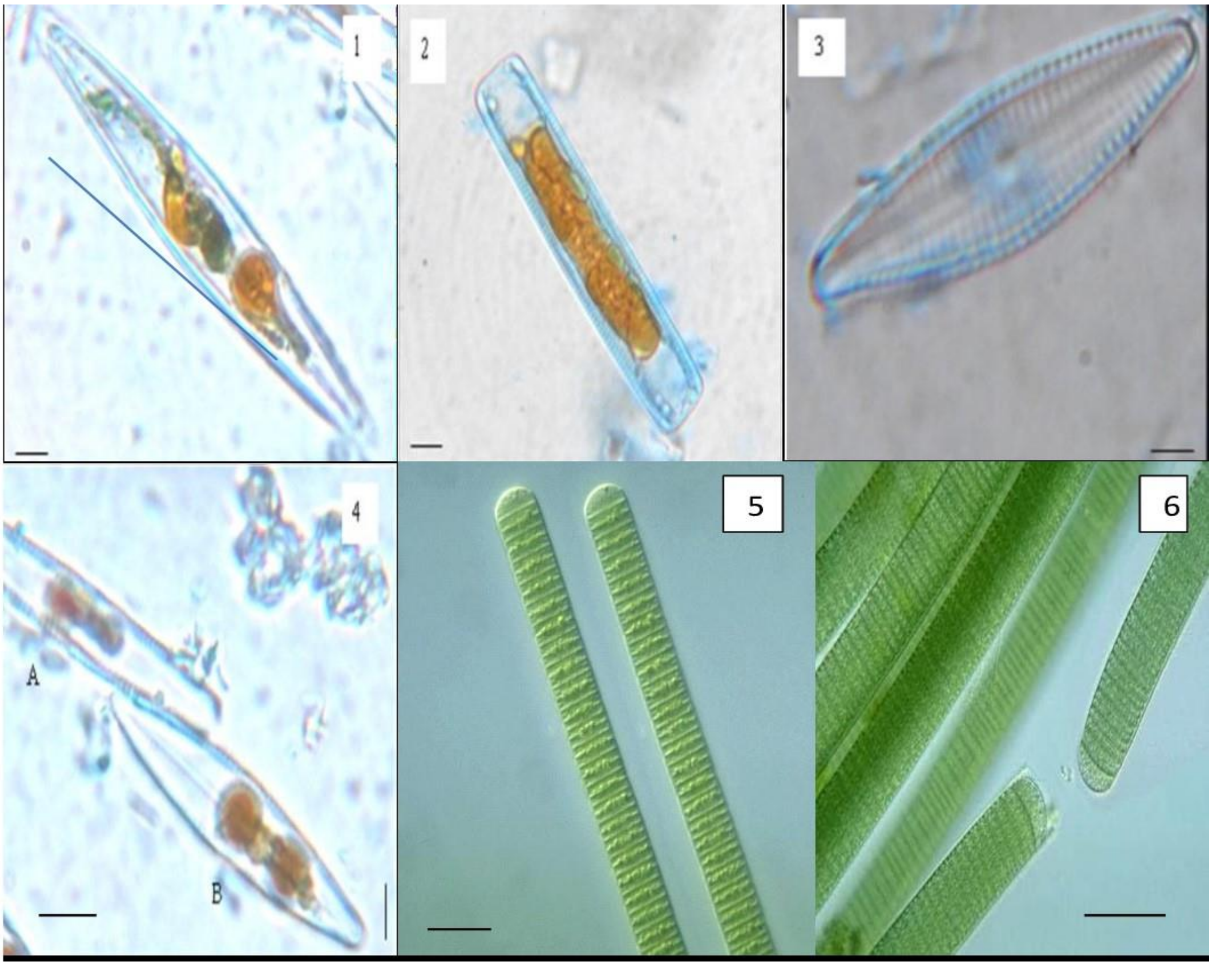

Fig. 1 - Fig. 6: showing algae associated with *Spirodela polyrrhiza* under light microscope (under 40 x objective).
Fig. 1: *Nitzschia sigmoidea* (Nitzsch) Smith, (Scale bar- 10 µ)
Fig. 2: *Anomoeoneis sphaerophora* Pfitzer. (Scale bar- 10µ)
Fig. 3: *Navicula gottlandica* Grunow (Scale bar- 10µ)
Fig. 4: *Gomphonema olivaceum* (Hornemann) Brebisson (Scale bar- 10 µ)
Fig. 5: *Oscillatoria jenensis* Schmid (Scale bar- 20 µ,)
Fig.6. *Oscillatoria sancta* Kutzing and Gomont. (Scale bar- 25 µ)

Table 3
Limnological parameters of the 15 Collection Sites of duckweeds in Summer of 2021.

Collection sites	pH	Turbidity (NTU)	TDS (ppm)	Nitrate (mg/L)	Phosphate (mg/L)
CSI	6.94±0.03	13.57±0.21	580±1.13	0.02±0.32	0.56±0.87
CSII	7.11±0.05	14.25±0.33	575±2.53	0.08±0.23	0.78±0.56
CSIII	7.96±0,40	13.75±0.23	582±2.98	0.03±0.12	0.71±0.87
CSIV	7.75±0,32	9.33±0,12	455±3.12	0.14±0.98	0.35±0.23
CSV	7.81±0.07	10.5±0.31	466±0.54	0.03±0.12	0.30±0.08
CSVI	7.54±0.06	6.75±0.12	452±3.22	0.08±0.21	0.45±0.12
CSVII	7.43±0.12	7.84±0.32	424±1.32	0.12±0.09	0.35±0.12
CSVIII	7.22±0.98	7.570±0.14	436±2.32	0.08±0.02	0.28±0.91
CSIX	8.22±0.08	10.4±0.33	394±1.33	0.06±0.13	0.36±0.08
CSX	8.52±0.12	11.5±0.54	355±2.12	0.04±0.05	0.98±0.12
CSXI	8.11±0.21	11.3±0.98	344±1.43	0.14±0.08	0.47±0.11
CSXII	7.58±0.12	11.52±0.43	374±1.23	0.1±0.12	0.77±0.43
CSXIII	7.61±0.32	11.82±0,65	384±0.66	0.06±0.12	1.047±0.12
CSXIV	7.54±0.09	10.7±0.34	390±0.56	0.09±0.11	0.95±0.43
CSXV	8.32±0.43	8.5±0.45	377±2.01	0.1±0.05	0.39±0.09

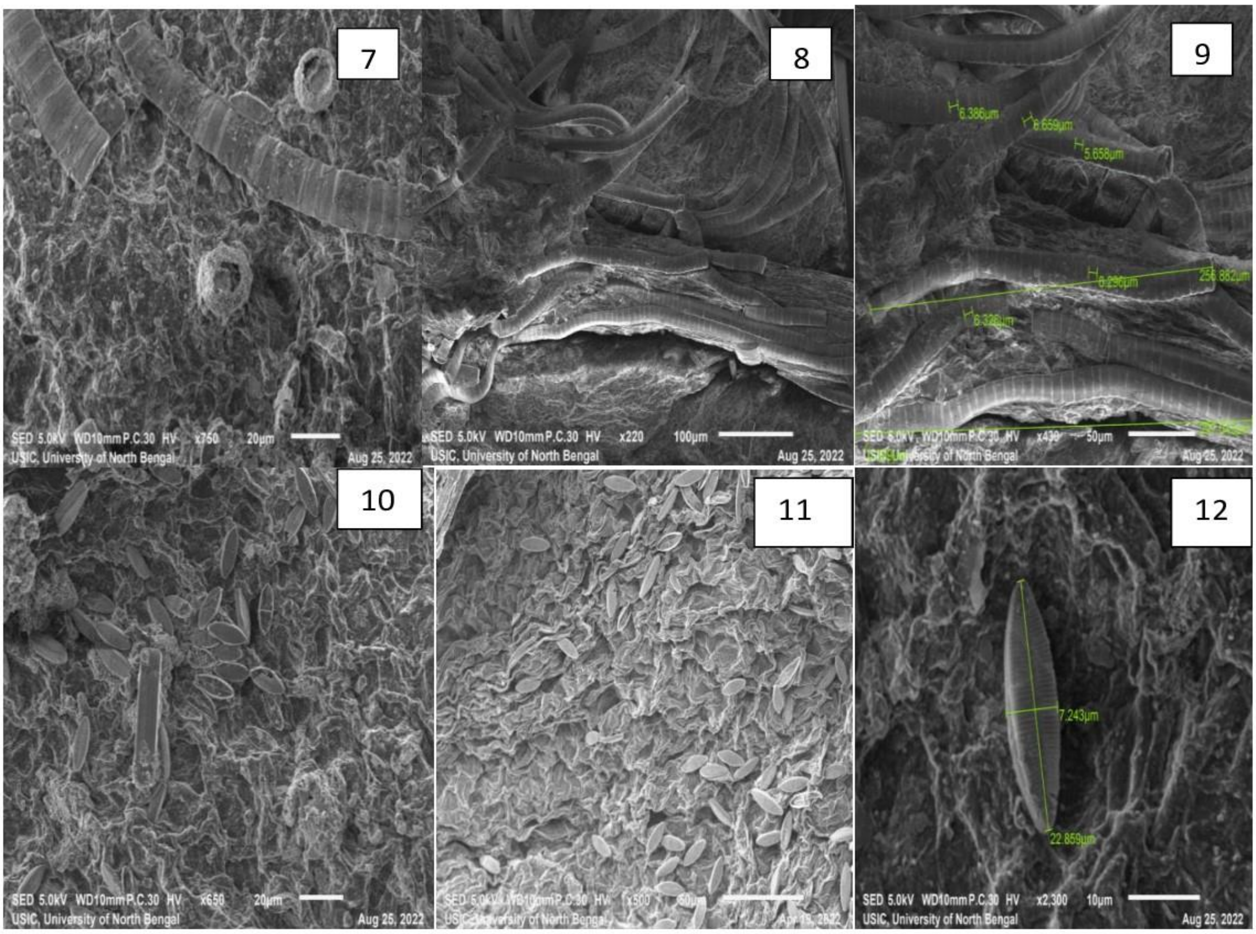

Fig. 7 – Fig. 12: Scanning Electron Micrographs of algae associated with the lower surface of the fronds of *Spirodela polyrrhiza*. Fig. 7: *Oscillatoria jenensis* Schmid, Fig. 8 and 9: *Oscillatoria sancta* Kutzing and Gomont, Fig. 10: *Nitzschia sigmoidea* (Nitzsch) Smith, Fig. 11: *Navicula gottlandica* Grunow, Fig. 12: *Gomphonema olivaceum* (Hornemann) Brebisson

Fig. 13: A-D- Glimpses of collection of fronds of *Spirodela polyrrhiza* (L.) Schleid from aquatic water bodies of Malda District

Table 4
Limnological parameters of the 15 Collection Sites of duckweeds in Winter of 2021

Collection sites	pH	Turbidity (NTU)	TDS (ppm)	Nitrate (mg/L)	Phosphate (mg/L)
CSI	6.23±0.23	13.66±.2.34	560±0.44	0.03±0.09	0.59±0.12
CSII	7.34±0.23	14.25±1.23	575±2.87	0.05±0.12	0.72±0.23
CSIII	8.16±0.45	14.11±0.76	515±2.12	0.04±0.21	0.74±0.12
CSIV	7.55±1.23	9.89±0.55	459±2.98	0.14±0.02	0.75±0.03
CSV	7.21±0.23	11.53±0.33	478±2.10	0.03±0.1	0.3±0.23
CSVI	6.94±0.44	6.75±0.34	412±2.13	0.09±0.12	0.49±0.21
CSVII	7.13±0.32	7.84±0.56	474±2.19	0.12±0.12	0.35±0.12
CSVIII	6.96±0.23	7.67 ±0.34	496±2.98	0.08±0.12	0.33±0.14
CSIX	8.22±0.87	10.4±0.56	394±1.23	0.06±0.02	0.36±0.19
CSX	8.52±0.78	12.5±0.22	355±0,98	0.08±0.12	0.38±0.12
CSXI	8.11±0.54	11.3±0.21	344±0.67	0.14±0.03	0.57±0.15
CSXII	7.58±0.34	10.52±1.2	414±3.12	0.1±0.12	0.67±0.33
CSXIII	7.11±0.45	11.82±1.02	389±1.32	0.06±0.12	0.947±0.11
CSXIV	7.67±0.23	12.7±0.87	410±5.12	0.05±0,23	0.989±0.13
CSXV	8.12±0.22	9.55±0.23	381±0.65	0.11±0.03	0.43±0.98

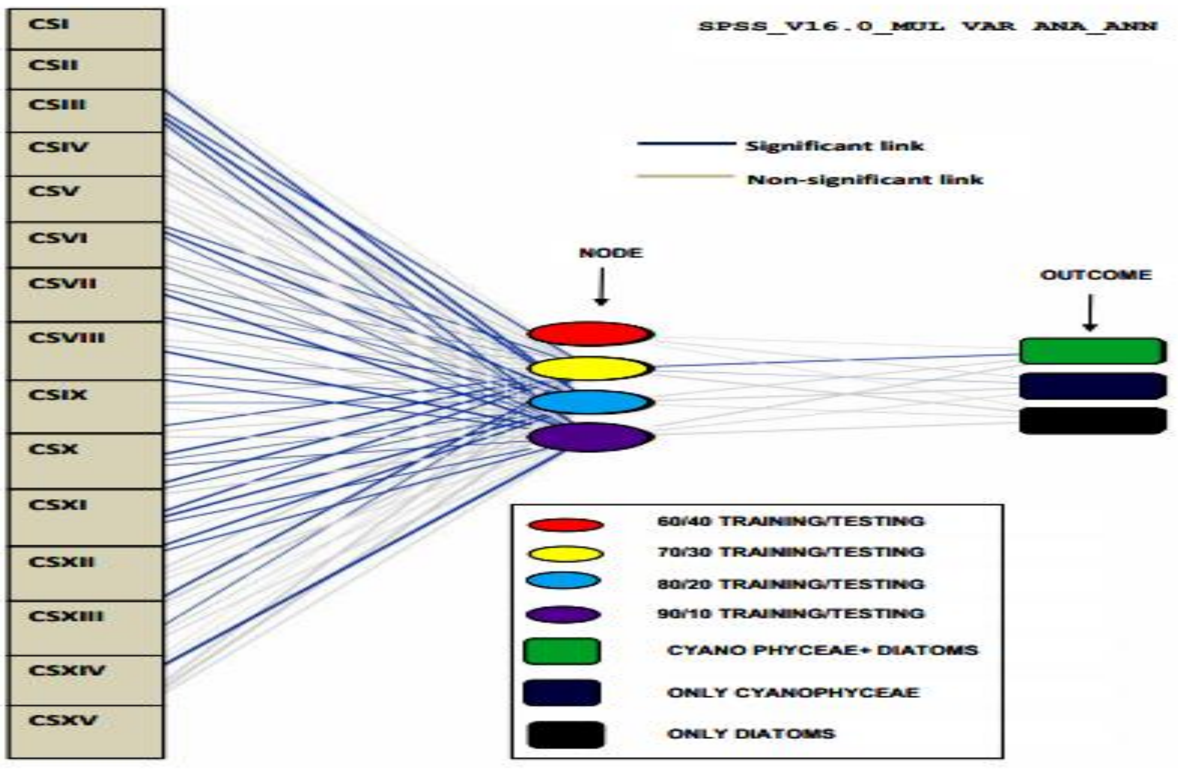

Fig. 14: ANN multivariate model showing influence pattern of independent variables on dependent variables through nodes as middle layer. Nodes represent training-testing allocation ratio that ultimately leads to significantly close-fit neural linkage between the two sets of variables.

The algae found to be associated with fronds of *Spirodela polyrrhiza* (L.) Schleid and identified as per Desikachary[6,7] which are as follows: i) *Nitzschia sigmoidea* (Nitzsch) Smith (Class- Bacillariophyceae), ii) *Anomoeoneis sphaerophora* Pfitzer (Class- Bacillariophyceae), iii) *Navicula gottlandica* Grunow (Class- Bacillariophyceae), iv) *Gomphonema olivaceum* (Hornemann) Brebisson (Class- Bacillariophyceae). v) *Oscillatoria jenensis* Schmid (Class- Cyanophyceae) vi) *Oscillatoria sancta* Kutzing and Gomont (Class- Cyanophyceae). Table 2 shows the association between the six algal genera and *Spirodela polyrrhiza* in all the 15 CS indicating their low and high frequency.

The six algal genera are given in figures 1-6. Their coexistence with fronds of *Spirodela polyrrhiza* was proved with help of Scanning electron micrographs (Figures 7-12). Fifteen collection sites were taken as the data source of respondent multivariate groups. The four intermediate nodes were formed by the software, representing 60/40, 70/30, 80/20 and 90/10 representing 60%-40%, 70%-30%, 80%-20% and 90%-10% segregation of respondents for training and then testing. Multivariate analysis was done by the software and link established with target variable in the form of significant and non-significant outcome. Blue lines represent significant link which was established with both Cyanophyceae and Diatoms.

70/30 segregation for training-testing shows uniformity in observation i.e. association is predictable even on the basis of 70% of the studied sites. It is a kind of commensalism where the cyanobacterial epiphytes use the duckweed for support, for protection against sunlight, as a source of carbohydrates and growth factors, but not symbiotic since both can survive separately in natural conditions. Algal colonies of *Gleotrichia*, *Calothrix*, *Nostoc*, *Anabaena* etc. are common in occurrence as epiphytes on large number of aquatic macrophytes[8]. Under direct sunlight, the nitrogen fixing ability of cyanobacteria decreases[5]. So it may be concluded that the cyanobacteria get a favourable environment for N_2 fixation under the lower epidermis of the fronds of *S. polyrrhiza*. The association may protect the greater duckweeds from oxidative stress under pathogen attack[16].

Conclusion

The close association of microalgae with the fronds of *S. polyrrhiza* in all the collection spots may be for the supply of nitrogen under natural conditions required for luxurious growth of duckweeds. Since two organisms share common ecological niche, so their co-cultivation can be possible. Diatoms species found attached with the fronds may be for their shelter as they do not influence the growth rate of the greater duckweeds under liquid culture conditions. Observations between duckweeds and blue green algae were reported in few cases from aquatic ecosystems of other tropical countries like Malaysia and Indonesian islands[11].

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