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Evidence for production of Indole-3-acetic acid from a fresh water cyanobacteria (*Oscillatoria annae*) on the growth of *H. annus*

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Abstract- Cyanobacteria are inexpensive to maintain with high growth rates and produce various biologically active substances like proteins, vitamins, carbohydrates, amino acids, polysaccharides and plant growth regulators. Thus they have the unique potential to contribute to productivity in a variety of agricultural and ecological situations. Indole acetic acid (IAA) is a natural auxin which is also synthesized in many species of non seeded plants, many bacteria, fungi and algae. The amino acid tryptophan is commonly regarded as the precursor for the biosynthesis of auxin in plants. By one pathway tryptophan is converted to indole pyruvic acid via a transaminase reaction, which requires a keto acid and pyridoxal phosphate in addition to the enzyme. Indole pyruvic acid is next decarboxylated to indole acetaldehyde in a reaction requiring a decarboxylase thiamine pyrophosphate. Either an oxidase or a dehydrogenase then oxidizes indole acetaldehyde to IAA. In this study, for the first time an attempt was made to confirm the presence of IAA in the extract of Oscillatoria annae instrumental methods of analysis and a field experiment was also conducted to analyze the efficacy of the extract.

Index Terms- Oscillatoria annae, indole – 3- acetic acid, HPLC, FTIR

I. INTRODUCTION

A growth regulator is a natural or synthetic organic compound that promotes, inhibits or qualitatively modifies growth and development of a plant. These compounds are biologically active at very low concentration and elicit response similar to those observed from plant hormones. Since most plant growth and development processes are regulated by natural plant hormones, these processes may be manipulated either by altering the plant hormone level or changing the capacity of the plant to respond to its natural hormones (Moore, 1989: Fullick et al., 2006).

Auxin is one of the plant growth regulator produced from the bacteria, fungi, cyanobacteria and plants. The auxins are classified based on the occurrence by natural source or synthetic (Nishida and Murata, 1996; Stirk et al., 1999).

Indole acetic acid (IAA) is a natural auxin which is also synthesized in many species of non seeded plants, many bacteria, fungi and algae. The amino acid tryptophan is commonly regarded as the precursor for the biosynthesis of auxin in plants (Sergeeva et al., 2002).By one pathway tryptophan is converted to indole pyruvic acid via a transaminase reaction, which requires a keto acid and pyridoxal phosphate in addition to the enzyme. Indole pyruvic acid is next decarboxylated to indole acetaldehyde in a reaction requiring a decarboxylase thiamine pyrophosphate. Either an oxidase or a dehydrogenase then oxidizes indole acetaldehyde to IAA (Pattern and Glick, 1996).

In some cases the basal application of biofertilizer or hormone may be drained into the water system. So the plant cannot utilize the basal fertilizer fully. Besides this, foliar fertilization has the advantage that translocation takes place directly into the plant. This application has been used as a means of supplying supplementary doses of minor and major nutrients, plant hormones, stimulants and other beneficial substances.

Cyanobacteria are inexpensive to maintain with high growth rates and produce various biologically active substances like proteins, vitamins, carbohydrates, amino acids, polysaccharides and plant growth regulators. Thus they have the unique potential to contribute to productivity in a variety of agricultural and ecological situations. The economically important host plants namely Helianthus annus L. selected for the study based on the usage and nutritive value for testing cyanobacterial extract as foliar spray.

II. MATERIALS AND METHODS

Cyanobacterial culture collection

The cyanobacteria were collected from various paddy (*Oryza sativa* L.) fields in Thanthonimalai, Karur District, Tamil Nadu, India.

Isolation

BG11 medium (Rippka *et al*., 1979) was used for isolation, identification and mass cultivation of cyanobacteria. Cyanobacteria *Oscillatoria annae* was isolated and purified by serial dilution technique.

One gram of cyanobacterial mat was homogenized and diluted in 100 ml (considered as 10^{-2}) of stock. From the stock one ml of cyanobacterial suspension was taken and it was transferred to 9ml of sterilized medium from 10^{-3} to 10^{-8} respectively. From each dilution 1 ml was trasferred and spread using L-rod on solidified BG11 agar medium in petriplates and incubated under controlled condition.

Culture maintenance and induction of auxin

Cyanobacterial cultures were maintained in BG-11 medium at 25±2 ^{0}C under 1500 lux light intensity with 14/10 D/L cycle for

7 to 15 days. For auxin production 10mg/100ml of tryptophan (precursor) was incorporated in BG11 medium **(Sergeeva *et al.*, 2002).**

Preparation of extract

Known amount of dried cyanobacterial strains were taken and ground with required amount of distilled water. Extraction was repeated until the cyanobacterial culture turned white residue. Then the extract was filtered through Whatman No.1 filter paper and the culture filtrate was dried for three days.

Extraction of IAA (Sergeeva *et al.*, 2002)

Oscillatoria annae was homogenized for 10 minutes and centrifuged at 5,000g for 20 minutes at 4ºC. The supernatent was acidified pH 2.8 with 1.0 M HCl.Acidified supernatant was extracted three times with ethyl acetate [1:3 v/v].Extracts were then evaporated under vacuum condition.The remaining aqueous fraction was adjusted to pH 7.0 with 1 N NaOH and it was extracted three times with water salurated n-butanol (1:3v/v). The extract collected from aqueous fraction dried under vacuum. The above No. 4 & 6 dried powder was dissolved with 80% methanol and filtered through membrane filter (pore size 0.45mm) and final weight was taken.

Bioassay for IAA-rice root inhibition assay (Mahadevan and Sridhar, 1996)

Oscillatoria annae extract (3ml) was added to 10ml 3% bacteriological agar. This solution was warmed in a water bath to melt the agar.Molten agar (5ml) was distributed into 10ml glass vials and autoclaved for120 ^{0}C for 15 min.To this molten agar without cyanobacterial extract / known concentration of IAA (10µg- 100µg) was added, which was maintained as control. Surface sterilized rice seeds were soaked in sterile water for 24hr at 25 ± 1^{0}C in dark. Seeds were spread on moist filter paper in petridishes and kept for another 24hrs in dark. The germinated seeds were transferred into sterile agar which containing cyanobacterial extract/ IAA standard. Seedlings were grown for 48hr in dark at 25 ± 1^{0}C. Primary roots were measured and the mean root length was measured after 48hr incubation. Standard curve was prepared by plotting logarithm of concentration µg/L compared with either % of growth or % inhibition over the control.

Estimation of IAA (Mahadevan and Sridhar, 1996)

The greyish blue spot corresponding to the authentic IAA was removed and it was diluted in 1ml of methanol. To this 2ml of Salper reagent was added drop wise with continuous agitation. Samples were then incubated in dark for 60min till a stable pink colour developed.The absorbance was measured at 565nm against a solvent reagent blank. A standard curve drawn from known concentration of IAA was used to quantify IAA present in the extract.

Detection of IAA by TLC (Mahadevan and Sridhar, 1996)

Cyanobacterial extract was separated on TLC plates coated with 250µm thickness of silica gel. 10µl of the extract and standard IAA (10µg/ml) were spotted on the plate. Isopropanol-ammonia-water (80:10:10 v/v) was used as mobile phase. After elution Erlich's reagent (2% p-dimethyl aminobenzaldehyde in 2N HCl in 80% ethanol) was sprayed on the chromatogram and dried in oven at 100 ^{0} C for about 8-10 minutes to detect the presence IAA.

Qualitative and quantitative estimation of IAA BY HPLC (Dobrev *et al.*, 2005)

The powdered extract were dissolved in 5ml of 1M formic acid to give 0.5-1 AU and the actual absorbance of solution were measured. The standard solution of a single compound was sequentially eluted and the absorbance of each elute was measured using spectrophotometer (SCHIMADZU 1700, Japan) with

Structural elucidation of IAA by FTIR Spectroscopy (Skoog *et al.*, 2004)

For a window of diameter 1.3cm, 160mg of spectroscopic grade KBr was taken in a previously cleaned pestle and mortar and it was ground thoroughly with 0.05-0.5mg of the dried cyanobacterial extract. The sample mixture was carefully placed into the pressing chamber of the mould in such a manner that it was held between the polished surfaces of the bottom and top pressing samples. Subsequently the chamber was attached to the vacuum line and vacuum pump was switched on . Initially a slight negative pressure was applied to compact the powder and then gradually increased the pressure to 15 mm Hg for 30 sec. Finally, the pressing force was enhanced to 1, 00,000 lb/in^2 or 10 -12 tons/in^2 for a period of 1-2 minutes. The pressure was carefully released and dismantled the samples.

The samples were analyzed using FTIR- 8400S SHIMADZU instrument with transmittance mode. The apodization was carried using Happ Genzel technique with 20 scans and of resolution of 2. A plot of absorption intensity (% transmittance) on the y axis against frequency on the X axis was made for individual samples with wave number ranging from (4000 cm^{-1} to 400 cm^{-1} upper).Transmittance (T) - The ratio of the radiant power transmitted by a ample to the radiant power incident of the sample.

Absorbance (A)- The logarithm to base 10 of the reciprocal of the transmittance. A - $\log_{10}$ (1/T) Frequency - The X axis is represented by scales.

1 Wave length - 2.5µ to 25µ (bottom)
2 Wave number - 4000 cm^{-1} to 400 cm^{-1} (upper)

III. FIELD STUDY

Mass cultivation

The mass cultivation of cyanobacteria was conducted in PVC tanks (sterilized) filled with sterilized BG11 medium. The *O. annae* culture was inoculated and it was incubated for 7-14 days at room temperature. After maturation the cyanobacterial mat were collected and dried for storage.

Field condition

Plot size: *Helianthus annus* L. - 11.15sqm
Design: Randomized Block Design
Variety: Ankur (*Helianthus annus* L.)
Treatments

C - Control (Without treatment)
C - Chemical fertilizer (Recommended dose)
T_1 - 0.01% of *O. annae* extract
T_2 - 0.02% of *O. annae* extract

T_3 - 0.03% of *O. annae* extract

Duplication - 25

Duration of crops: *Helianthus annus* L. - 90 days
Recommended dose of chemical fertilizer (NPK kg/ha):
Helianthus annus L. - 40:20:20

***O. annae* extract as foliar spray**
The seeds of *Helianthus annus* L. were treated with the extract of *O. annae* (0.01%, 0.02% and 0.03%). After germination, the seedlings were transplanted to respective fields for further treatment with the extract. The 1st, 2nd and 3rd doses of the *O. annae extract* to *H. annus* extract were applied on 10th, 30th and 60th day. Mean time the morphological characters and biochemical characters were analyzed and tabulated at 30th, 60th and 90th day.

Analysis of parameters
Morphological Parameters
1 Length of the root, shoot and diameter of the stem
2 Fresh and dry weight of the root and shoot
3 Leaf stalk, leaf numbers, leaf area, and weight of the leaves
4 Number of branches, internodal length, number of internodes, Number of seeds, weight of seeds per head, surface diameter of the head (in *Helianthus annus* L.)
5 Weight of the fruit, diameter of the fruit, plant biomass and productivity of the crop plant.

Biochemical Parameters
Estimation of Chlorophyll (Arnon, 1949)
1g of fresh leaf was ground in mortar and pestle with 80% cold acetone with a pinch of $CaCO_3$ to prevent pheophytin formation. The homogenate was centrifuged at 2500g for 10 min. The supernatant was made up to a known volume with 80% cold acetone. Optical density was measured against acetone as blank in spectrophotometer (Shimadzu 1700, Japan) at 645nm and 663nm. Chlorophyll *a, b* and total chlorophyll were calculated using Arnon's formula.

Estimation of carbohydrates (Yemm and Willis, 1954)
Dried plant materials (100mg of leaves, fruits or seeds) were exhaustively extracted with 70% (v/v) ethanol and the extract was evaporated in vacuum. Evaporated residue was dissolved in distilled water to a final volume of 1ml, and which was kept in a water bath for 30 ºC. Anthrone reagent (5ml) was added to the test (1ml) and standard solution. To the test solution, 0.5ml of H_2SO_4 was added and it was cooled for 5min.Tubes were loosely fitted with corks heated for 5min. and then cooled in water bath. The absorption spectra were determined in a spectrophotometer (Shimadzu 1700 Japan) at 600nm. The measurement of test solutions and reagent blanks were made against water as a reference.

Estimation of Protein (Lowry *et al.*, 1951)
Fresh plant material 500mg was washed in distilled water. It was ground in 0.1M potassium phosphate buffer (pH 7.0) using a pestle and mortar under cold condition and the homogenate was centrifuged at 7000g for 10 min; the supernatant was decanted and was used for protein estimation. Protein in the supernatant was precipitated by adding equal volume of 5 % Trichloroaceticacid. The precipitate was removed by centrifugation at 7000g for 15min and dissolved in a known amount of 1N NaOH to give the protein solution.From the protein solution, 0.5ml was pipetted out and the total volume was made upto 4ml with distilled water. To each tube 5.5ml of the alkaline mix (reagent C) was added and mixed well and allowed to stand at room temperature for 15min. From the reagent D, 0.5ml was pipetted and added into each tube and mixed rapidly after each addition. The tubes were left for 30min and the development of blue colour was measured at 650nm (Shimadzu 1700. Japan). A standard graph was plotted by using bovine serum albumin V (Sigma) and the protein content in the sample was estimated with the help of the standard graph.

Estimation of total lipids (Tomlinson and Rich, 1969)
Lipids are heterogenous group of biological compounds that are insoluble in water but soluble in ether, chloroform and other organic solvents. The hydrocarbon of lipid contributes the hydrophobic nature. Lipids are generally bound to forms proteins in biological samples and cannot be efficiently extracted with non polar solvents alone. In such cases lipids are extracted with a mixture of chloroform and methanol and are easily separated by this procedure.
One gram of plant sample was homogenized with 10ml of chloroform/methanol (2:1 v/v) mixture in a mortar and pestle. The homogenate was filtered through cheese cloth. The residue was re-extracted with 10ml of chloroform methanol mixturean and the extracts were pooled. The crude lipid extract was made upto a volume of 20ml with chloroform / methanol mixture.To the crude extract, equal volume of distilled water was added in a separating funnel. The content was mixed thoroughly by vigorous shaking and allowed to stand for the separation of chloroform layer from aqueous phase. The chloroform phase was withdrawn by a vacuum dryer and weight (mg/g) was observed.

Estimation of nitrogen (Jackson, 1958)
Dried leaf sample (100mg) was taken in a boiling test tube. To this 3ml of concentrated sulfuric acid was added and this content was boiled for 15min. Few drops of perchloric acid was added during boiling and was finally made upto50ml using distilled water. From this 2ml of sample was taken in microjeldhal flask along with 4ml of 40% NaOH. The liberated ammonia was collected in a conical flask which contains 5ml of 2% boric acid. After the colour change (pink to blue) in boric acid to titrated against N/70 H_2SO_4until the appearance of pink colour. The blank was also titrated without adding sample, finally the total nitrogen content in the sample was estimated by the following formula.

$$\% \text{ of Nitrogen} = \frac{\text{Titrant value of sample} - \text{Titrant value of blank X 50 X Strength of N/70} H_2SO_4}{2}$$

Estimation of phosphorus (Fiske and Subba Rao, 1925)
1g of leaf sample was digested with 5ml of perchloric acid. From this, 1ml of digested sample was pipetted out into a test tube. To

this 0.4ml of ANSA and 1ml of molybdate solution I and 1ml of molybdate solution II were added and made upto 10ml using distilled water. The contents were mixed well and the color developed was read spectrophotometrically (Schimadzu 1700,Japan) at 660nm after 20min. The phosphorus content of the unknown sample was calculated by plotting against standard graph.

Analysis of micronutrient in soil

The micronutrients and macronutrients in the soil sample were analyzed at the Tamil Nadu Agriculture University, Madurai. Soil microflora were also analyzed in soil before and after the treatment of *O. annae* extract

IV. Results and Discussion

Cyanobacterial culture collection

Mixtures of cyanobacterial (blue green in colour) culture were picked up randomly from various paddy (*Oryza sativa* L.) fields in Thanthonimalai of Karur District, Tamil Nadu, India.

Isolation

Cyanobacterial strains were separated, isolated using spread plate technique and twenty different strains from the mixture were isolated and were being maintained in BG-11 medium and the strains were designated as 1-20. Survey of occurrence of cyanobacteria in rice fields showed that out of 2213 samples collected from different region of India showed 33% of samples contain nitrogen fixing cyanobacteria. These organisms grow together with the rice plants and form patch of thick yellowish /brownish/blue green mats on water surface **(Venkataraman, 1981)**.

Detection of IAA in *O. annae*

Extraction 0f IAA

IAA was extracted from ***O. annae*** using organic solvents and analyzed for the presence of PGR activity by performing rice root inhibition assay.

Bioassay for IAA

In order to confirm the plant growth regulating activity in different concentrations from 0.01%-0.05% **(Fig.2)** of *O. annae* extract the root inhibition assay was performed in *Oryza sativa* L. Among all the concentrations of the cyanobacterial extract, the highest root growth was obtained in 0.01% of the treated plants. But, the growth rate slowly decreased from 0.02% to 0.05% of cyanobacterial extract treated plants. Reduction in growth by higher concentration of cyanobacterial extract was well supported by the report of **Raghava and Murty (1988)**.

Report showed that auxin like compounds were released by about 38% of the free-living cyanobacteria as compared to 83% of the symbiotic cyanobacterial isolates. Endogenous accumulation and release of IAA was confirmed immunologically (ELISA) using an anti-IAA antibody on 10 of the Salkowski-positive strain and the chemical authenticity of IAA was further verified by chemical characterization using gas chromatography-mass spectrometry. Evidence on the production of hormonal substances by cyanobacteria has come primarily from treatment of rice seedlings with algal cultures or their extracts **(Gupta and Lata, 1964)**.

A significant increase in the length of the coleoptile and radicle was observed due to whole cell extracts. A similar influence on the growth of roots and shoots has also been shown **(Gupta and shukla, 1969)**. Auxins like growth promoting substances was detected in both *Nostoc muscorum* and *Hapalosiphon fontinalis* by bioassays in rice seedlings **(Misra and Kaushik, 1989)**. *Cylindrosporum muscicola* can synthesize vitamin B and inter convertible auxin like substance which stimulates root growth of rice seedlings **(Venkatram and Neelakantan, 1967)**. **Sergeeva *et al.*, (2002)** reported the possible role of IAA in cyanobacteria in general and their interactions with plants.

Detection of IAA by TLC

Using thin layer chromatography (TLC) IAA and its various derivatives present in dried cyanobacterial extract were separated. Among the three spots. R_f value of the one spot coincided with that of standard IAA (R_f 0.83). The other two spots corresponding to R_f value of 0.54 and 0.67cm were to be indole derivatives like indole pyruvic acid and indole lactic acid. The test sample was also analyzed for the presence of glutamic acid and cytokinin, but corresponding spots did not appear in cyanobacterial extract chromatogram and this method was supported by **Joseph and Bernard (2005).**

Estimation of IAA

The *O. annae* extract containing IAA was quantified using colorimetric method and the estimation revealed that 1g of cyanobacterial extract contains 5.2µg/g of IAA **(Fig.1)**. Production of IAA by *O. annae* was well supported by **Tsavkelova et al., (2006)** who reviewed the ability of prokaryotes and eukaryotes like Pseudomonas, Anabaena, Fusarium and Saccharomyces to synthesize growth stimulating phytohormones. The presence of auxin like growth promoting substances were shown in both Nostoc and Hapalosiphon and their quantities were 3.76 and 4.48mg/g respectively (**Misra & Kaushik, 1989).**

Structural elucidation of IAA by FTIR

The IR spectrum of cyanobacterial sample shows the peak at 3389.32 cm^{-1} which indicated **(Fig. 3 -7)** the presence of NH stretch. The peaks at 1100.252, 1207.83 and 1223.75cm^{-1} indicated the presence of C-C stretch. The peak at 1701.9cm^{-1} indicated the C=O stretch. The peak at 3092.49cm^{-1} indicates the presence of aromatic stretch. Comparison of the functional groups in standard IAA and the cyanobacterial extract confirmed the presence of IAA in the cyanobacterial sample. The present investigation are in full accordance with the research findings indicated by **Krystyana and Cohen, (1986)** where in an amide conjugate of IAA was isolated and purified from *P. vulgaris*. Since the present experiment results revealed the presence of IAA in cyanobacterium *O.annae,* the study has been applied to field trials for improving the crop yield.

Field study

Mass Cultivation

Mass cultivation of *O. annae* was carried out in PVC tanks and *O. annae* in the form of thick greenish mat floats on the medium was harvested and dried for storage.

Field Condition

Experiments were carried out in different areas of varying soil types showing acidic pH and are well ploughed and marked before planting the seedlings.

Analysis of Parameters

Morphological Parameters

Various morphological growth parameters of *Helianthus annus* L. of the experimental plant such as shoot length, fresh and dry weight of the shoots and roots, number of internodes, number and mean area of leaves significantly increased in 0.01% concentration of cyanobacterial extract treated plants **(Fig. 8-10)** when compared to control plants. Whereas, in higher concentrations like 0.02%, 0.03% and chemical fertilizer showed less growth when compared with 0.01% concentration *O.annae* extract sprayed at different concentration on experimental plant *H. annus* showed that, concentration of 0.01% significantly increased germination percentage. The response of cyanobacterial extract treatment varied with different concentration and shows 0.01% of extract enhanced hypocotyl and epicotyl length of the three experimental plants. The increase in shoot length by cyanobacterial extract could be due to increased cell elongation (**Bonner, 1950 and Sharma, 1988**). Plant growth regulating hormones can induce major changes in the development of plant tissues, such as general increase in cell division and cell elongation or specific developmental changes such as the induction of new roots **(Davies, 1987)**.

Interestingly, it was observed that in the lower concentration (0.01%) of cyanobacterial extract enhanced growth and in the higher concentrations (0.02% and 0.03%) of cyanobacterial extract shows shunted growth of *H. annus*. Growth inhibition at higher concentration of cyanobacterial extract was well supported by **Raghava and Murty (1988)** who reported that higher concentration of IAA showed inhibitory effect on plants.

Biochemical Parameters

The increase in biochemical parameters were more pronounced in 0.01% *O. annae* extract treated plant, *H. annus* L.. **(Fig. 11 - 14)** than the other treatments (untreated, chemical treated, 0.02% and 0.03% of *O. annae* treatment).

Chlorophyll contents increased in 0.01% *O. annae* extract treated plants than the control plants. Chemical fertilizer treated plants showed moderate amount of chlorophyll content than the 0.02% and 0.03% concentrations and control plants. **Mahla *et al.*, (1999)** reported that application of NAA and mixtalol NAA spray increased chlorophyll contents in leaves, there by increased photosynthetic efficiency over control. This might have led to provide more assimilates for better modulation. The increase in chlorophyll content as a result of growth regulator application might be because of better uptake of nitrogen, magnesium and other elements which are involved directly in the synthesis of chlorophyll **(Tagade *et al*., 1998; Sachan and Sarayya 1999; Kim and Pyo 1970; Mishra *et al*., 1976) (Fig. 27, 31 and 35)**.

Cohen, 1986 reported that cyanobacteria possess all known phycobiliproteins (phycocyanin, phycoerythrin, phycoerythrocyanin, allo-phycocyanin) which are commercially valuable. A commercial process of phycocyanin production from open scale cultivation of marine cyanobacterium *Phormidium valderianum* BDU30501 was developed **(Sekar and Subramanian, 1988). Haeley, 1968** showed that the spectrum of carotenoids in *Anabaena variabilis* and three species of *Phormidium* showed ß-carotene as the major pigment. Similarly, carotenoid composition of *Anabaena flosaquae* and three other species of *Phormidium* also showed the presence of ß-carotene as the major carotenoids in all species **(Hertzberg *et al*., 1971)**.

Aruna and Kannaiyan (1998) reported that inoculation of rice seedlings with sugarcane waste and PU foam immobilized. *Anabaena azollae* and *Anabaena variabilis* have accumulated higher total chlorophyll, protein and amino nitrogen compared to the inoculated rice seedlings. **Uma and Kannaiyan, 1995** stated inoculation of immobilized cyanobacteria PU foam improved the total carbohydrate, protein, amino nitrogen and chlorophyll content of the seedlings significantly. Also they reported higher growth, nitrogenase activity, ammonia excretion and heterocyst frequency than free-living cultures.

The variations in presence of protein, carbohydrate, lipids, nitrogen and phosphorus content in leaves quantified. It showed that 0.01% treated plant sample increase content of the various parameters and this might be due to uptake of more nutrients from the soil. Thus the biochemical parameters showed components decrease in values with increasing concentration of cyanobacterial extract. The hormones are responsible for increasing the physiological and metabolic activities in the plant tissue as a consequence of which there is an increase in uptake of nitrogen from the soil and its further assimilation for biosynthesis of protein **(Singh and Randhawa, 1969)**. These reports fully supported the increase of nitrogen and phosphorus content in leaves in 0.01% treated plants.

Soil Analysis

The micro and macronutrient contents decreased after the treatment of *O. annae* extract treated plants **(Table: 1)**. Here, all the treatments showed the utilization of NPK, Zn, Cu, Fe, Mn and $CaCO_3$ by the plants when compared with control and other treatments. Thus 0.01% cyanobacterial extract treated plants showed higher utilization of micro and macronutrient than the other concentration of cyanobacterial extracts treated plants. It may due to hormone application causes increase in physiological and metabolic activities of plant which resulted in higher uptake of nutrients from the soil. The observed higher nitrogen content in plant tissue in growth regulator treated plant might be due to the same element **(Tagade *et al*., 1998)**. The heterotrophic population in the soil increased after the treatments. It could be due to the translocation of IAA on the plants which directly influences the growth and there by the plant releases the root exudates into the soil that are utilized by the heterotrophic micro flora. Report shows that death of algal biomass is most frequently associated with soil desiccation at the end of the cultivation cycle and algal growth has frequently resulted in a gradual build up of soil fertility with a residual effect on succeeding crops. The pattern of distribution of total organic and mineral nitrogen studies in inoculated and un inoculated plots indicated a higher mineral nitrogen content and a low mineralisable index of N in the inoculated plots **(Chopra and Dube, 1971)**. **Singh *et al*., 1981** reported that organic manure which contained phosphorous beside nitrogen increased the soil organic phosphorous content leading to increased phosphorous availability and consequently higher uptake by rice plants. Further reports support that organic carbon, total nitrogen and available phosphorous of the soil were increased due to

application of *azollae* and other organic manures indicating that they released their nitrogen and phosphorous in the soil after decomposition **(Subudhi and Singh, 1980)**.

Yield

The variation in seed yield due to various concentrations of cyanobacterial extract treatments was statistically significant. The application of cyanobacterial extract in lower concentration (0.01%) showed maximum yield when compared to all other treatments and control and the yield obtained in 0.01% treatment was significantly superior over chemical fertilizer in *H. annus* L. **(Fig. 15)**.

On application of plant growth regulators, there was an increase in growth parameters, biochemical contents and yield when compared to control plants. It may be because of apparent increase of photosynthesis due to comparatively large volume and surface of the plant **(Kulkarni, 1977)**. Similar finding of increase in yield of groundnut by IAA was reported by **Shamsunder and Vittalrao, 1980**. **Rajula and Padmadevi (2000)** showed increase in germination percentage, shoot, root length and biochemical content like protein, carbohydrate, amino acid in the seedling of *Helianthus annus* L. grown in effluent blended with cyanobacteria.

Application of exogenous growth regulating substances to improve crop productivity was extensively supported by the report of **Pando and Srivastava (1985)**. **Kumar *et al*., 1980** observed enhanced fresh weight, leaf number, root and stem length in lady's finger (*Abelmoschus esculentus*) after addition of culture filtrate of nitrogen fixing cyanobacteria. Hence the application of cyanobacterial strain no.6, namely *Oscillatoria annae* extract as foliar spray in 0.01% concentration showed better results in terms of morphological, biochemical and yield parameters when compared to other concentrations of *O. annae* extract, control and chemical treatments.

Hence the application of cyanobacterial strain No.6 namely *O.annae* extract as a foliar spray in 0.01 % conc. showed better result in terms of morphological, biochemical and yield parameters when compared with other concentrations of *O.annae* extracts, control and chemical treatments.

V. FIGURES, GRAPHS AND TABLES

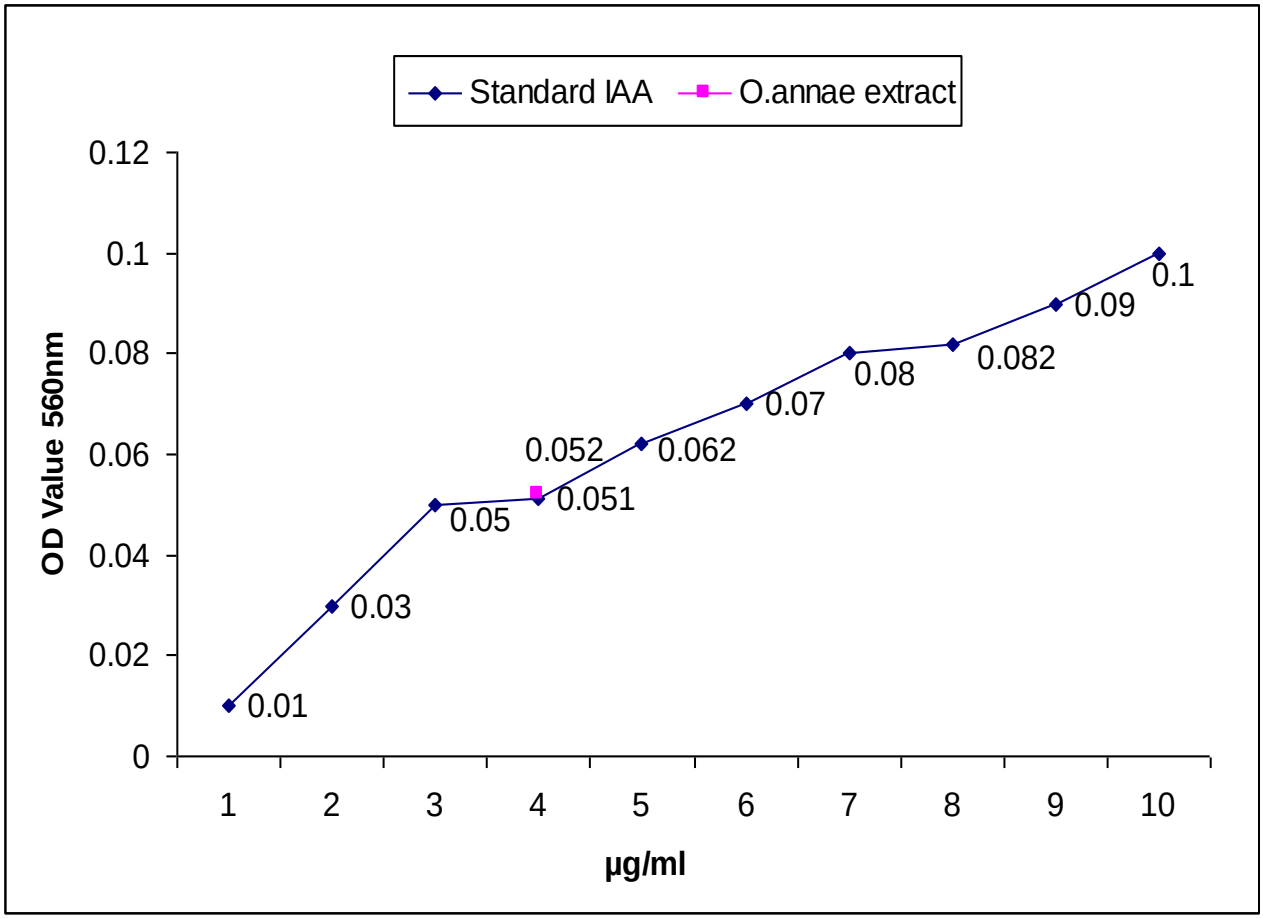

Figure 1: Estimation of IAA

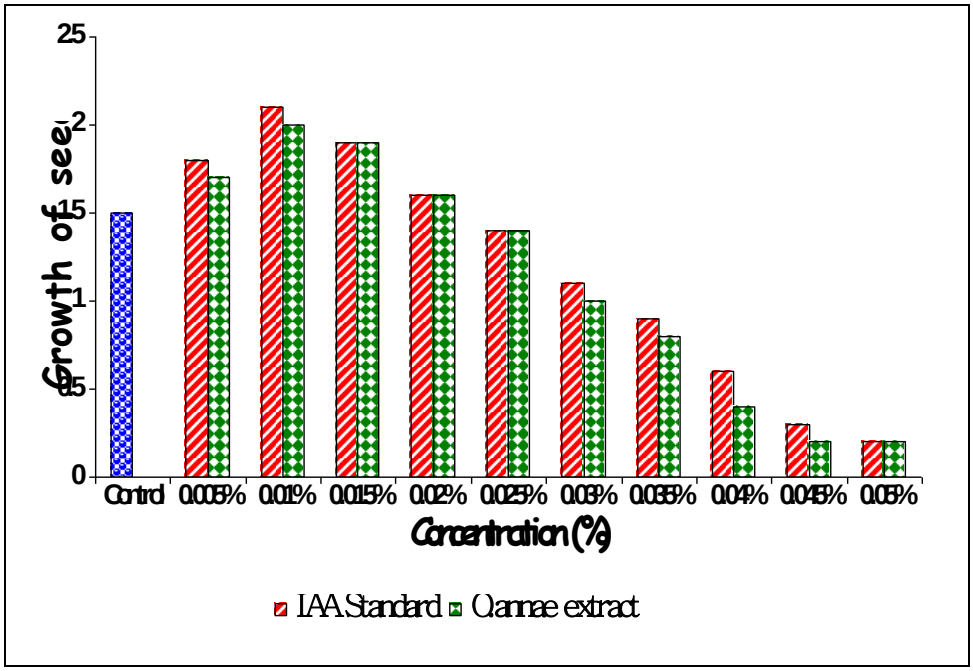

Figure 2: Bioassay for IAA (Root inhibition assay)

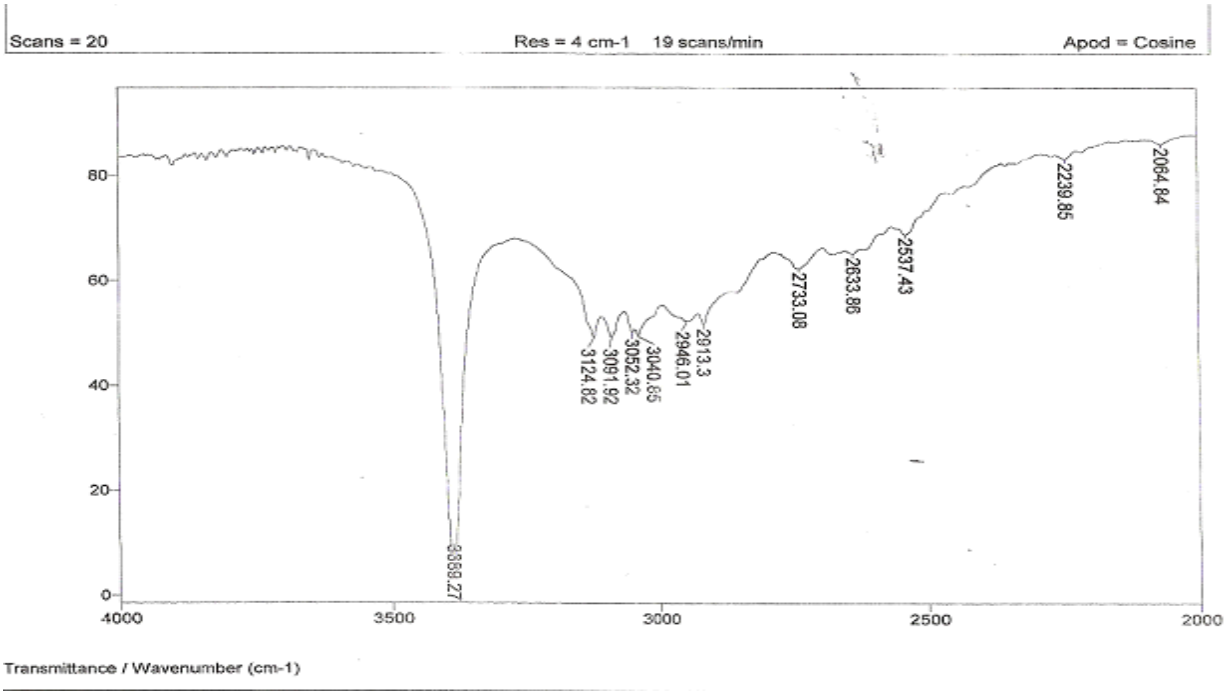

Figure 3: Characterization of standard IAA by Infra Red Spectra (from wavenumber 4000 – 2000)

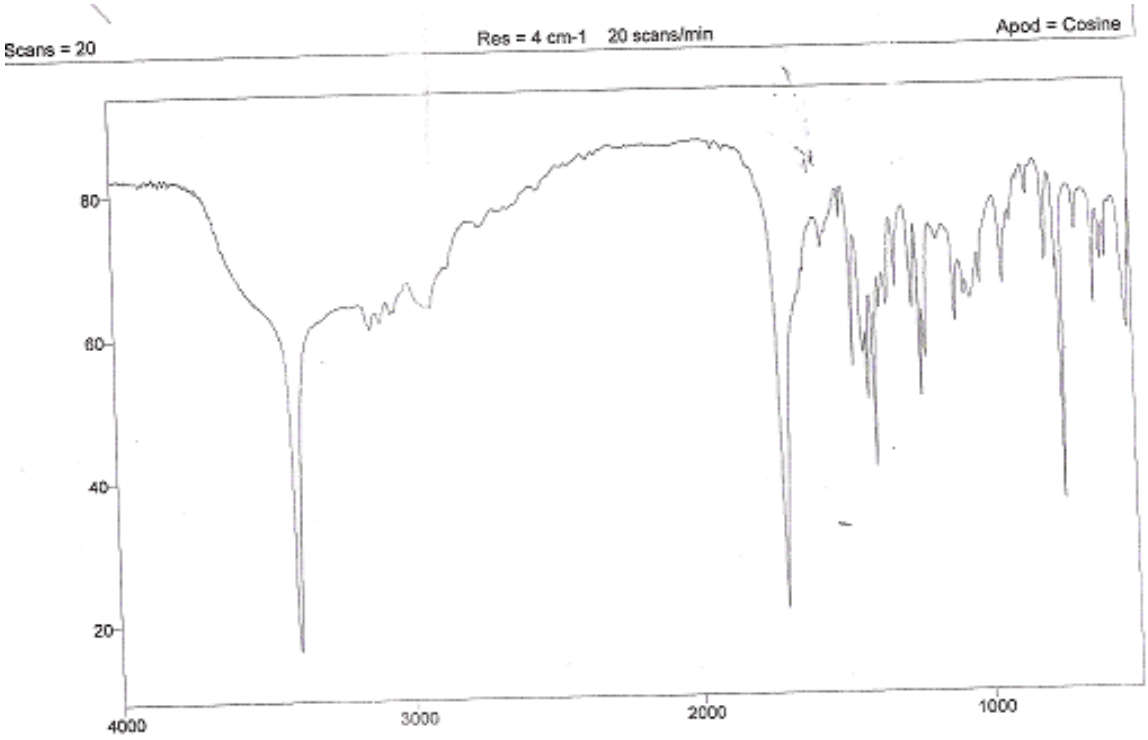

Figure 4: Characterization of IAA in ***Oscillatoria annae*** extract by Infra Red Spectra (from wavenumber 4000 – 500)

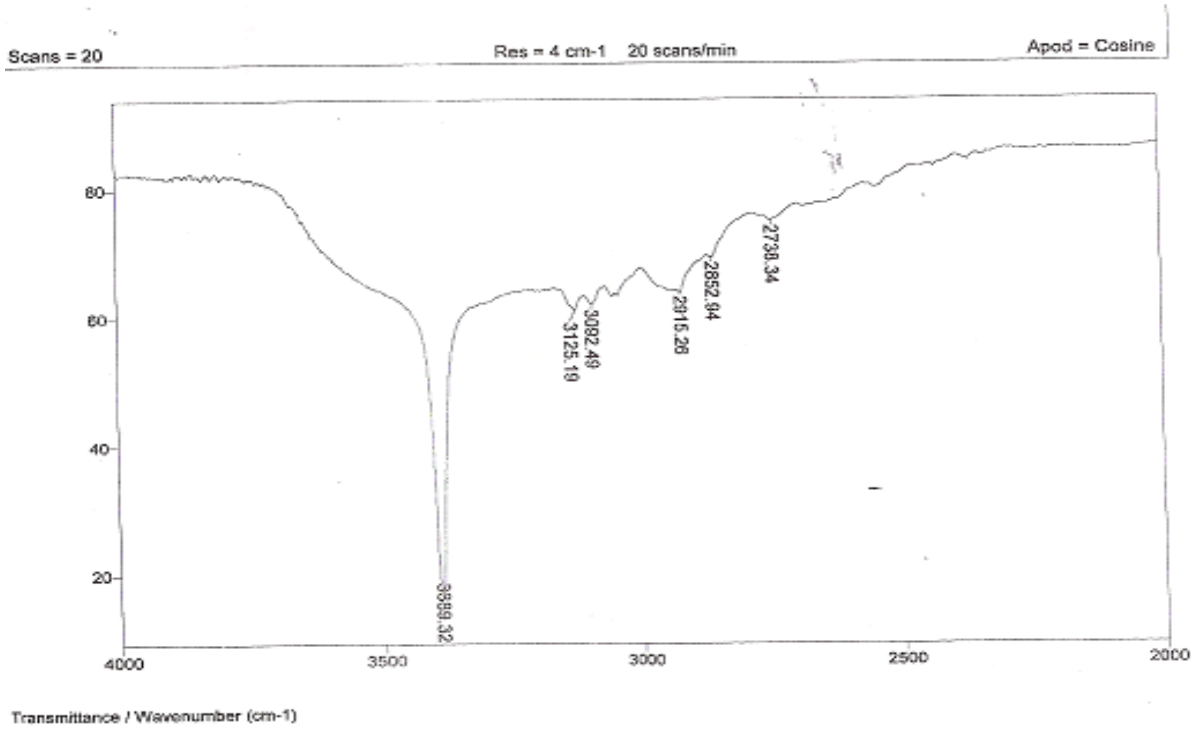

Figure 5: Characterization of IAA in ***Oscillatoria annae*** extract by Infra Red Spectra (from wavenumber 4000 – 2000)

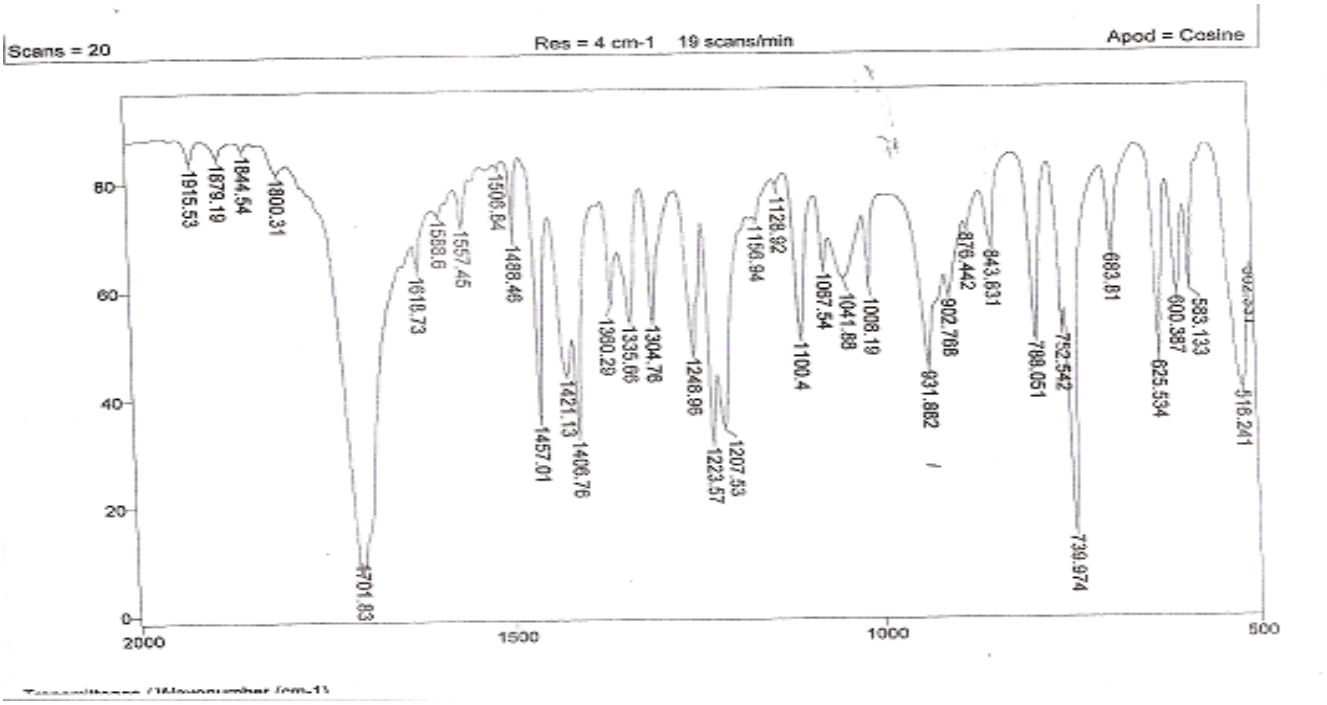

Figure 6: Characterization of standard IAA by Infra Red Spectra (from wavenumber 2000 – 500)

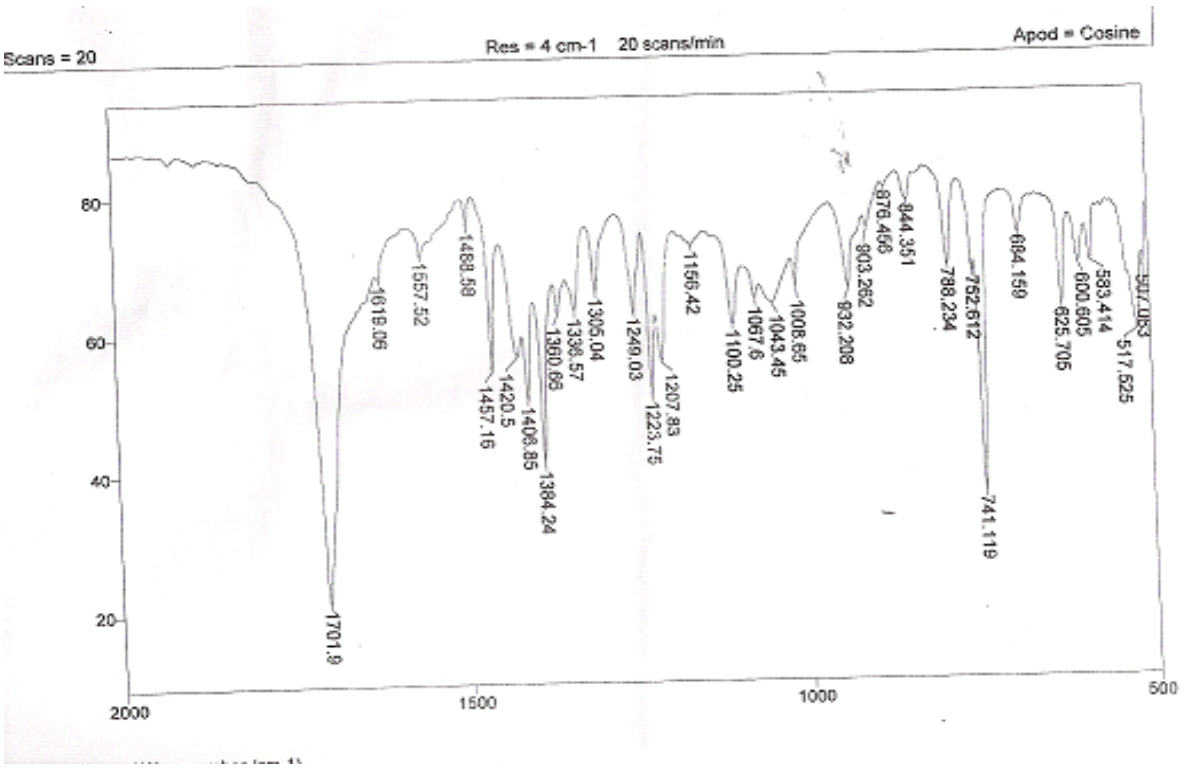

Figure 7: Characterization of IAA in ***Oscillatoria annae*** extract by Infra Red Spectra (from wavenumber 2000 – 500)

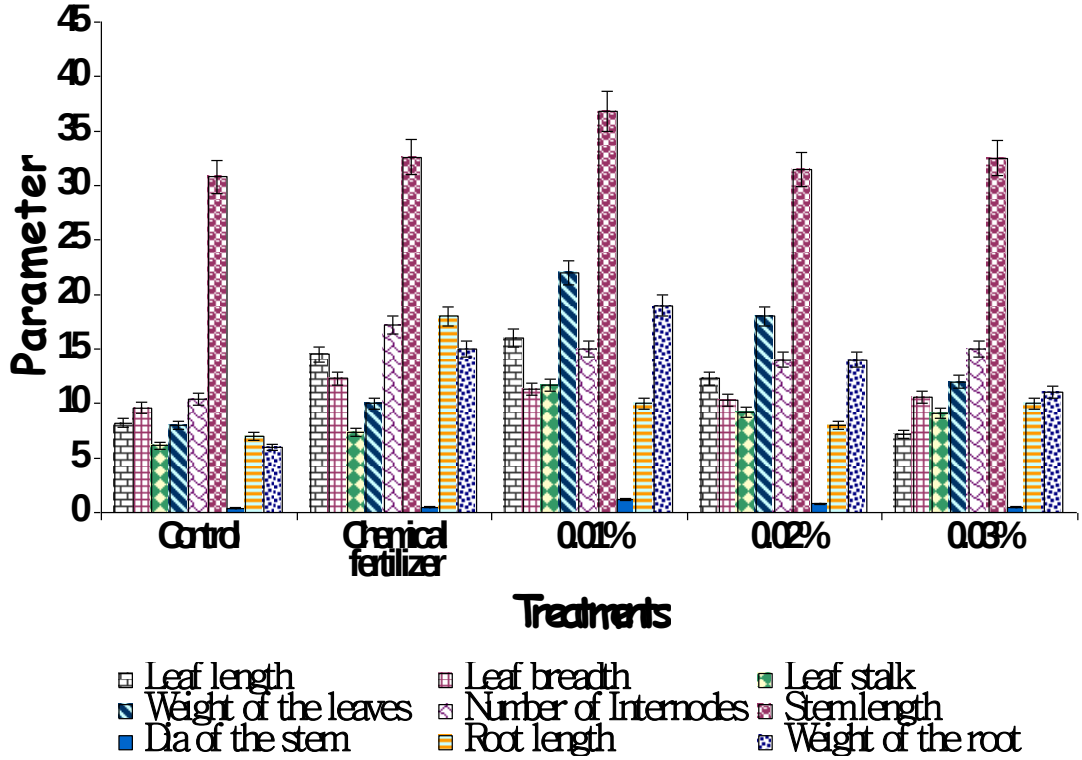

Figure 8: Impact of cyanobacterial extract on different morphological characters of ***Helianthus annus*** L. (30th day)

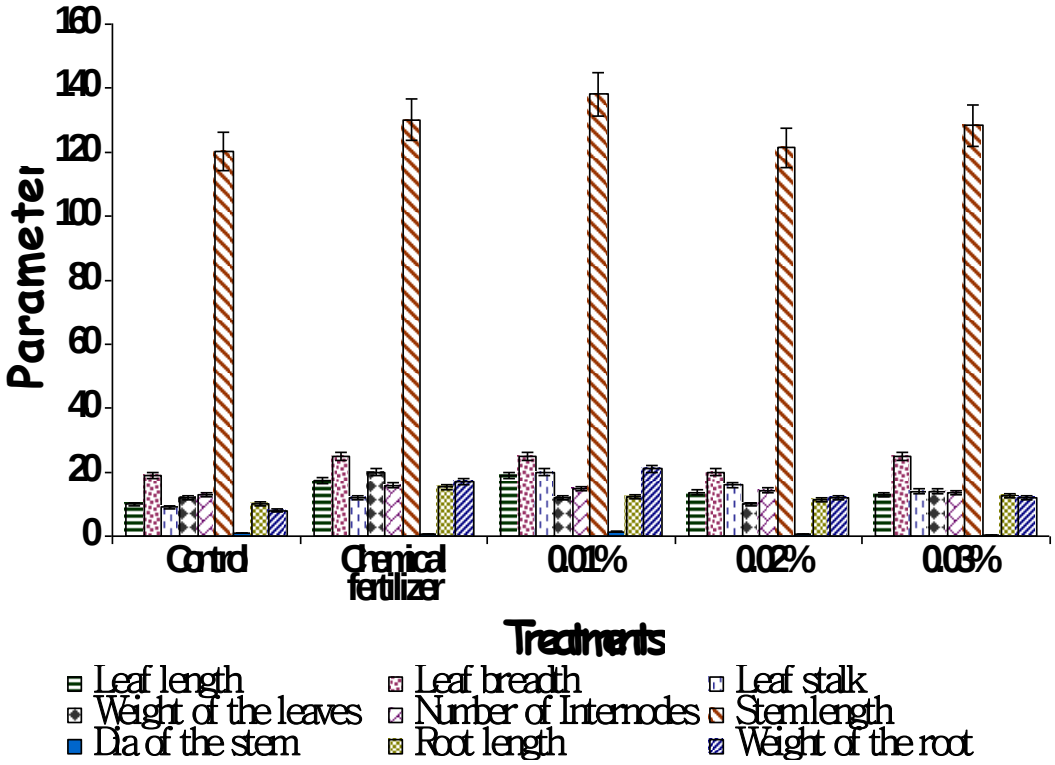

Figure 9: Impact of cyanobacterial extract on morphological characters of ***Helianthus annus*** L. (60th day)

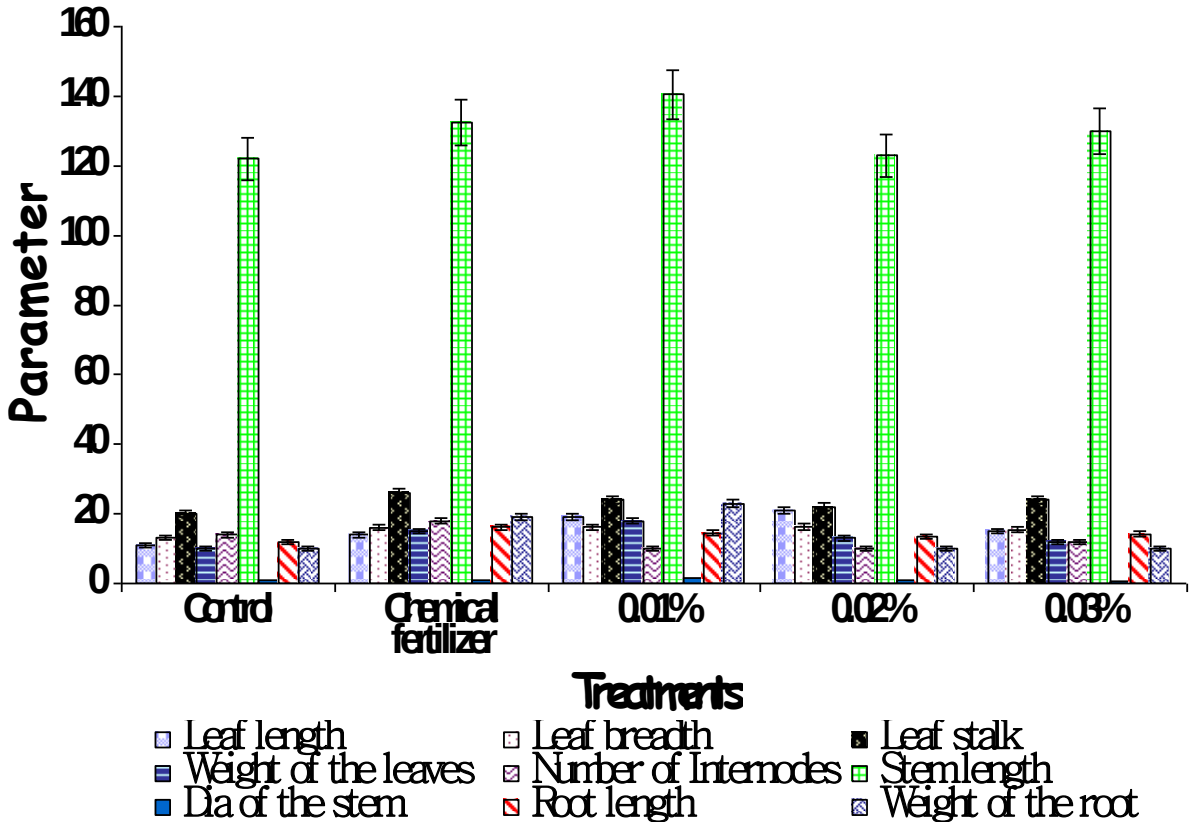

Figure 10: Impact of cyanobacterial extract on morphological characters of ***Helianthus annus*** L. (60th day).

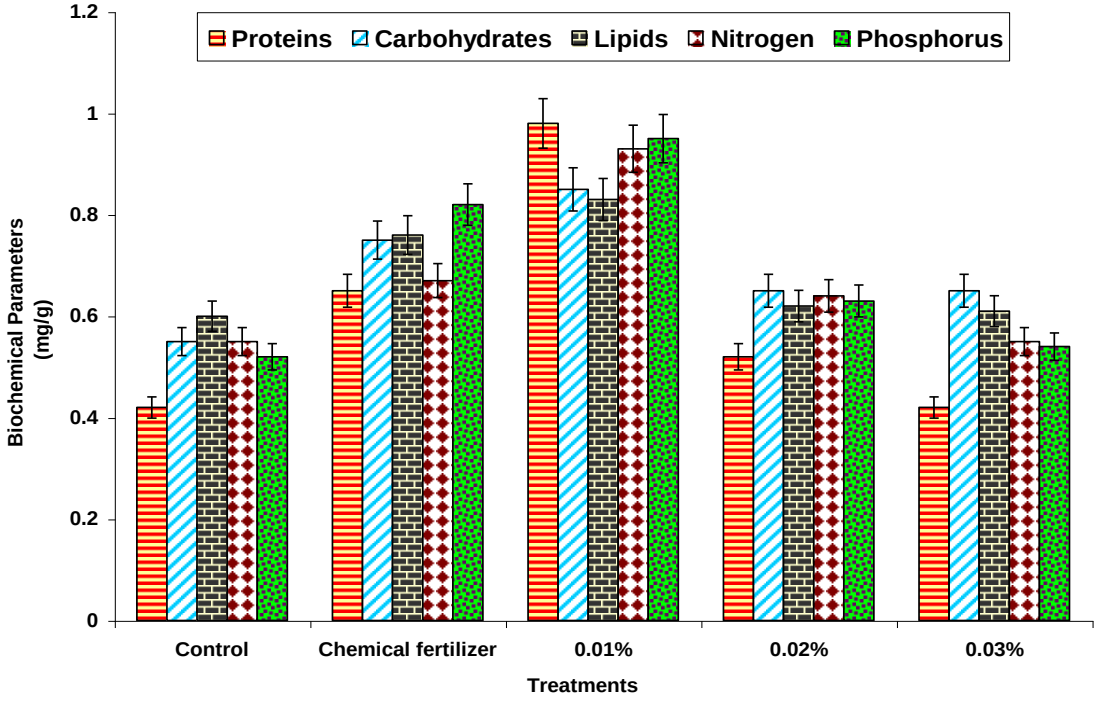

Figure 11: **Effect** of ***O. annae*** extract on the different biochemical characters in ***Helianthus annus*** L. (30th day)

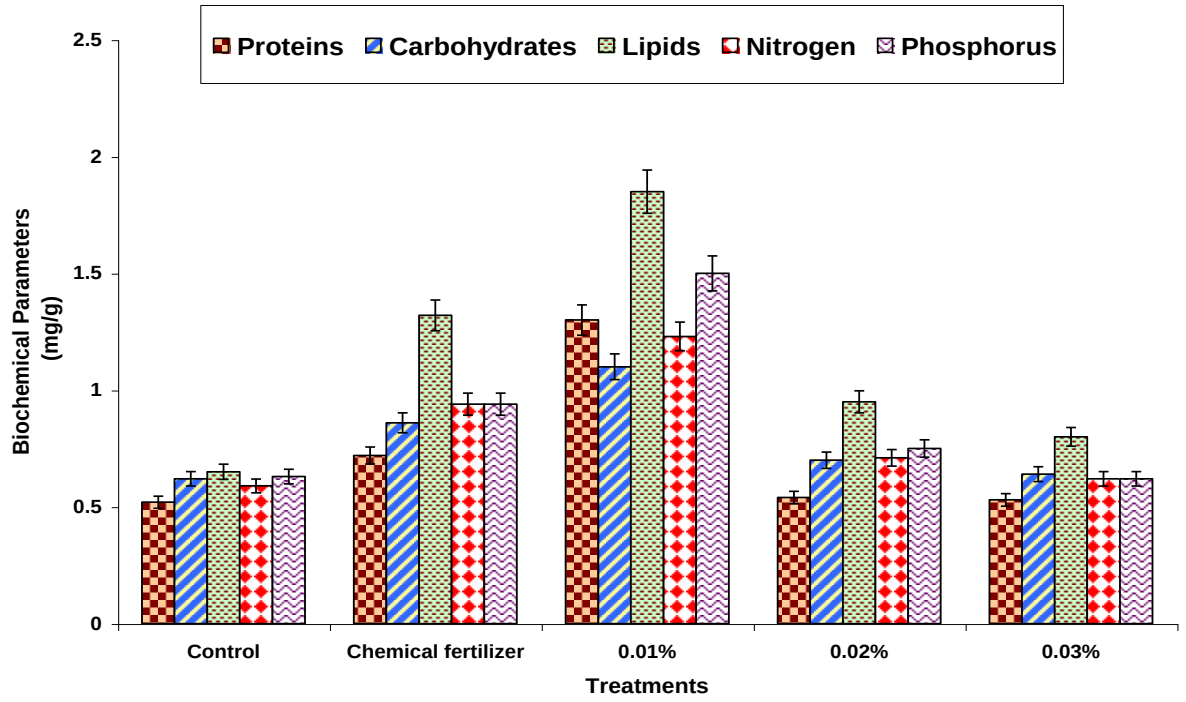

Figure 12: **Effect** of ***O. annae*** extract on the different biochemical characters in ***Helianthus annus*** L (60th day)

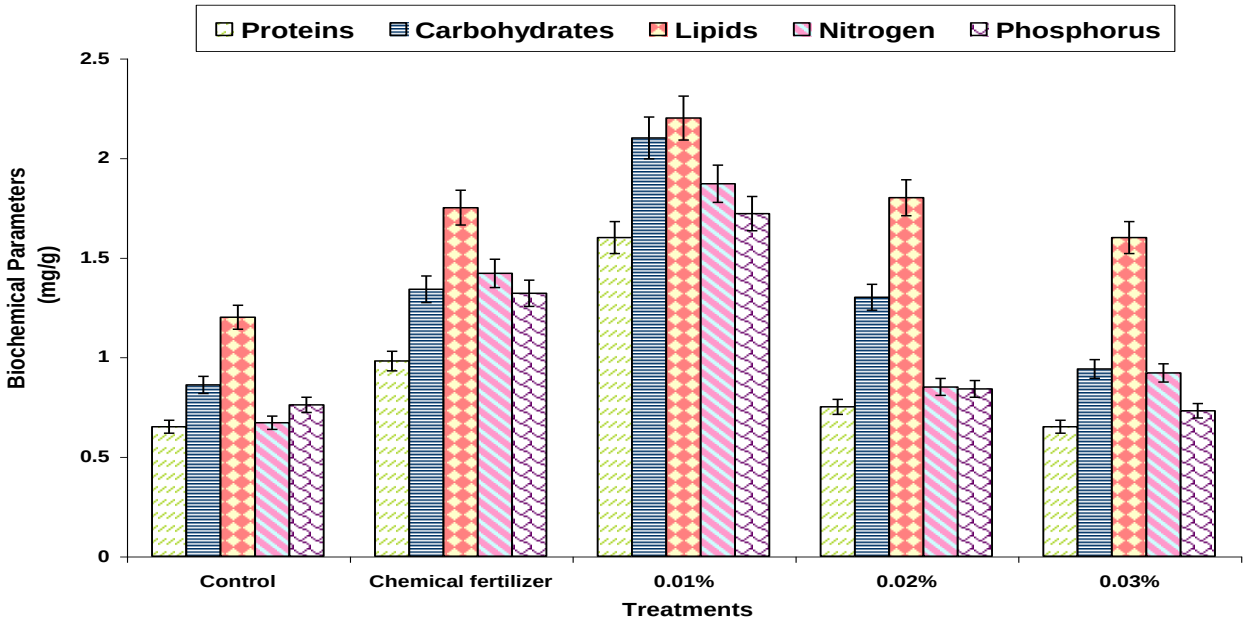

Figure 13: Effect of *O. annae* extract on the different biochemical characters in *H. annus* L. (After harvesting)

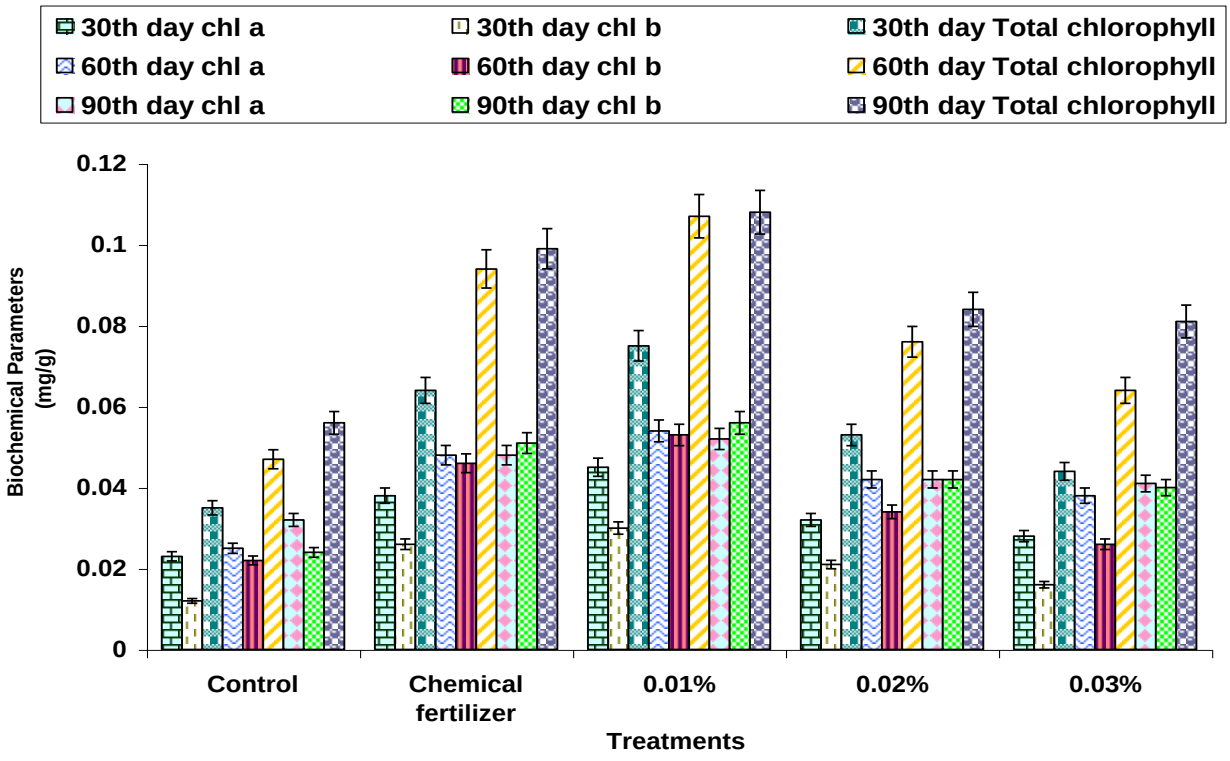

Figure 14: Effect of cyanobacterial extract on chlorophyll content (dry wt) of *H. annus* L. on 30th, 60th and 90th day.

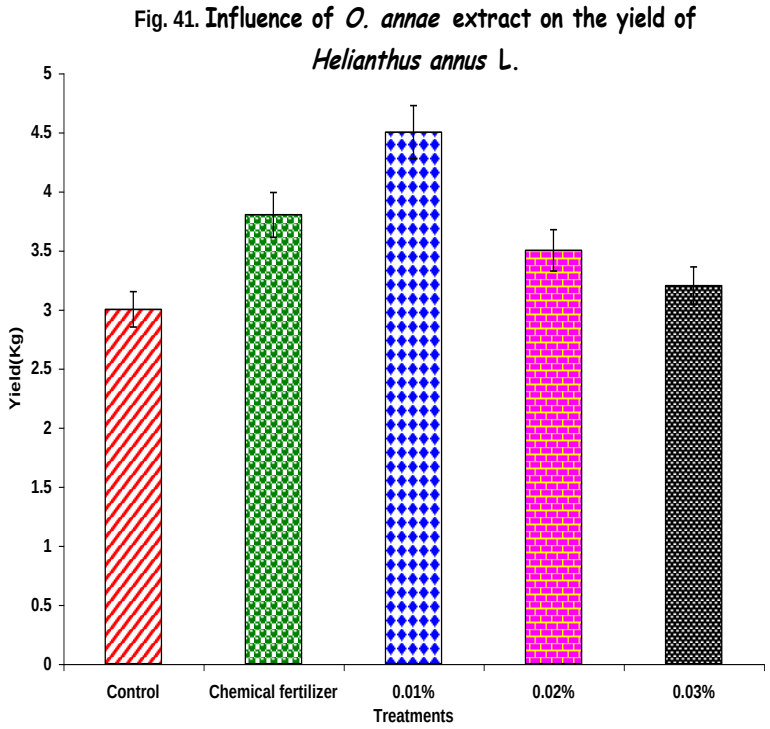

Figure 15: Yield of *Helianthus annus* L.

Table 1: Soil Analysis (before and after the treatment of O. annae extract on Helianthus annus L.)

Treatments	**Sample (Black Soil)**	**Nature of the soil**		**Macronutrients (kg/ha)**			**Micronutrients (kg/ha)**				**Other nutrients**	**Total bacterial count (cfu\g)**
		pH	**EC dSm^{-1}**	**N**	**P_2O_5**	**K_2O**	**Zn**	**Cu**	**Fe**	**Mn**	**$CaCO_3$**	
Before treatment	Blank Soil	8.5	0.05	59	7.6	133	0.16	-	1.94	1.1	-	0.86×10^6
After treatment	**Control**	**8.5**	**0.04**	**48**	**7.0**	**110**	**0.15**	**-**	**-**	**0.6**	**-**	**$0.75x10^6$**
	Chemical fertilizer	**8.5**	**0.04**	**45**	**6.8**	**90**	**0.14**	**-**	**-**	**0.6**	**-**	**0.89×10^6**
	0.01%	**8.0**	**0.04**	**46**	**7.2**	**100**	**0.16**	**-**	**-**	**0.6**	**-**	**$0.78x10^6$**
	0.02%	**8.5**	**0.04**	**47**	**7.8**	**105**	**0.16**	**-**	**-**	**0.6**	**-**	**$0.69x10^6$**
	0.03%	**8.5**	**0.04**	**50**	**7.4**	**92**	**0.14**	**-**	**-**	**0.5**	**-**	**$0.82x10^6$**

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