Single cell fertilizer (SCF): Evidence to prove that bio-molecules are potent nutrient for plant growth

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Abstract Fertilizers of various kinds are used for the cultivation of crop plants for hyper production of plant based food materials.. The study used bio-molecules made in a bacterial cell .The experimental results showed tremendous effect on plant growth.. These cellular molecules were made by treating the bacterial cells with lysozyme and protenase K.. The wet/weight was increased in multiple folds compared to that of control sets . The fold of increase was 4.79 for rice, 2.77 for wheat, 1.89 for gram and 1.89 for pea when bacterial cellular molecules were used as fertilizer.

Key words Fertilizer, Bio-molecules, bacteria, plant growth, pollution

Introduction

Inorganic and organic molecules of various types are used for the production of crop plants even though most of them cause serious environmental problems (Sen et al . 1981 ; Sen et al. 1982). Bio-fertilizers are now gaining popularity because these materials are eco-friendly however these materials have slower effect in increasing production of crop plants. Similarly, vesicular

arbusticular mycorrhizae and their use evolved in this field because they have great roles to improve and ensure soil fertility (Allen 1995). Repeated cropping, lack of crop rotation, heavy use of inorganic fertilizers are the major reasons of depletion of soil fertility in agricultural land. Moreover, use of bio-fertilizer made with free living nitrogen fixers, symbiotic nitrogen fixers, cyanobacteria, ferns of various kinds those are able to fix nitrogen, are now in practice even though they have slow effect on plant growth (Slater and Somerville 1979) The advantages are that these bio-fertilizers restore soil structure and maintain soil texture. Combinations of inorganic and organic molecules with high NPK value that provide suitable C: N ratio in agricultural land is also in practice. This study used bio-molecules of various types that were made in a non-pathogenic bacterium. These molecules are biodegradable, eco-friendly, less expensive with additional advantages compare to that of conventional fertilizers.

Materials and methods:

Seeds of various crop plants

Germinated seeds of gram (*Cicer orientinum* L) pea (*Pisun sativum* L) Rice (*Oryza sativa* L) and wheat (*Triticum estivum* L) were used in this investigation.

Growth of seedlings

The germinated seeds were transferred into Petri plates containing soil or absorbent cotton wool of equal amounts. The soil was collected from the agricultural field of the District, Burdwan, West Bengal, India. It was alluvial type of soil. The seedlings were distributed on these cultivation matrix with the addition of 10.0 ml of distilled water and requisite amount of

fertilizer. Two control sets were made. Each fertilizer was added in separate experimental set in requisite amount.

Harvesting of the grown plants

These plantlets were cultivated in the laboratory conditions in an open air for many days. Every 5th day was the harvesting day. Five plant lets were taken out from the experimental sets.. The wet-weight, length of roots and shoots were measured using an electronic balance and cm scale. Every day 10.0 ml of distilled water was added to the soil or cotton matrix. Every 5th day was the day of addition of fertilizer in requisite amount.

Addition of fertilizers

Urea, ammonium sulfate, sodium nitrate, glycine were used as the common inorganic and organic fertilizers. Bacterial cellular molecules were made following a technique. These molecules were added as the bio-molecules. The organic and inorganic fertilizers were added with the amount of 50 , 100 , 200 mg/set respectively. The addition of fertilizer was done every alternate 5th day. The bio-molecules were added with the amount 2.5 ml, 5.0 ml and .7.5 ml respectively. The number of cells were 10^{9} .ml^{1}.

Bacterial strain and growth

Preparation of bacterial culture *Escherichia coli* HB 101 strain was grown in Luria broth containing 1% Tryptone, 0.5% yeast extract, (Difco , Detroit ,USA) 0.5% NaCl, pH 7.2 (Lederblog and Cohen, 1974).. The bacterium was grown at 37 ^{0}C for 24hrs using a shaker incubator.

Preparation of Bio-molecules

The overnight bacterial culture was incubated at 37^{0}C with 10 mg of lysozyme (100ml culture / 10 mg of lysozyme) for 6 hrs. in shake condition. It was incubated further with 10 mg of protenase K (100 ml culture/ 10 mg protenase K) in shake condition at 37 ^{0}C for 8 hrs.(Maniatis et al 1982 This cell lysate of *Escherichia coli* (HB 101) was used as the source of bio-molecules (Watson, 1980) The approximate amounts of various molecules were inorganic ions 20, carbohydrates 60, lipids and fats 40, proteins 300, DNA 20, RNA 120 μg.ml-$^{-1}$. Other molecules were free amino acids and free nucleotides and nucleosides 80 ng . ml $^{-1}$

Results and Discussions

Fertilizers of various kinds have accelerating effect on growth and development of crop plants. Bumper cropping is done using many inorganic fertilizers with high NPK value. The experiment utilized various inorganic, organic and bio-molecules as fertilizers. The wet-weight of these plants was increased significantly using all these molecules (table 1-4) . The shoot and root parts of these plants were increased in all experimental sets.

Table I Effect of several molecules on the growth of rice

Type of Molecules	**Type of Plant**											
	***Oryza sativa* L**											
	Wet-weight (g)				**Length (cm)**							
					Root				**Shoot**			
	Days											
	I	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**
Sodium nitrate	**0.135**	**0.25**	**0.53**	**0.70**	**-**	**4.5**	**15.4**	**16.0**	**-**	**3.7**	**8.0**	**8.2**
Urea	**0.140**	**0.25**	**0.55**	**0.71**	**-**	**3.5**	**8.0**	**8.2**	**-**	**3.7**	**5.9**	**7.8**
Ammonium Sulfate	**0.150**	**0.30**	**0.60**	**0.65**	**-**	**12.5**	**7.0**	**8.5**	**-**	**6.3**	**6.5**	**6.6**
Glycine	**0.155**	**0.20**	**0.40**	**0.54**	**-**	**6.0**	**5.2**	**5.8**	**-**	**3.6**	**6.2**	**6.4**
Biomolecules	**0.155**	**0.25**	**0.58**	**0.91**	**-**	**4.6**	**5.7**	**9.0**	**-**	**4.5**	**6.2**	**8.2**
Control	**0.150**	**0.15**	**0.18**	**0.19**	**-**	**5.5**	**5.6**	**5.8**	**-**	**2.5**	**7.7**	**7.8**

All experimental results were the arrange of five individual and separate experimental results. 1, Beginning of the experiment. II, after five days of experiments, III, after 10 days of experiments. IV, after 15 days of experiment; -, indicates the length of root and shoot were not measured. In this experiment cotton (absorbent) was used as the cultivation matrix. The concentrations of various molecules were as per materials and method.

Table 2 Effect of various molecules on the growth of wheat

Type of Molecules	***Triticum aestivum* L**											
	Wet-weight (g)				**Length (cm)**							
					Root				**Shoot**			
	Days											
	I	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**
Sodium nitrate	**0.495**	**0.85**	**0.87**	**0.88**	**-**	**12.2**	**12.5**	**12.8**	**-**	**6.5**	**9.0**	**15.3**
Urea	**0.480**	**0.75**	**0.77**	**0.90**	**-**	**11.9**	**12.1**	**13.6**	**-**	**6.1**	**9.1**	**14.2**
Ammonium Sulfate	**0.421**	**0.70**	**0.72**	**0.95**	**-**	**9.5**	**10.3**	**11.6**	**-**	**3.4**	**7.5**	**11.9**
Glycine	**0.500**	**0.65**	**0.66**	**0.98**	**-**	**9.0**	**9.5**	**10.5**	**-**	**8.5**	**15.0**	**17.0**
Biomolecules	**0.455**	**0.80**	**0.92**	**1.30**	**-**	**13.5**	**15.5**	**18.5**	**-**	**15.5**	**17.8**	**19.5**
Control	**0.459**	**0.95**	**0.46**	**0.47**	**-**	**7.8**	**6.0**	**6.5**	**-**	**4.7**	**5.8**	**6.8**

Experiments were done as per the method as described in table 1

Table 3 Effect of various molecules on the growth of gram

Type of Molecules	**Type of Plant**											
	***Cicer Orientinum* L**											
	Wet-weight (g)				**Length (cm)**							
					Root				**Shoot**			
	Days											
	I	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**
Sodium nitrate	**1.80**	**2.75**	**3.15**	**3.60**	**-**	**8.0**	**12.5**	**13.5**	**-**	**3.0**	**12.0**	**14.5**

Urea	**1.68**	**2.95**	**3.21**	**3.70**	**-**	**7.2**	**9.0**	**9.5**	**-**	**2.5**	**16.0**	**23.0**
Ammonium Sulfate	**1.77**	**2.60**	**2.68**	**2.78**	**-**	**13.5**	**15.5**	**16.2**	**-**	**4.5**	**11.0**	**17.0**
Glycine	**1.95**	**2.90**	**3.55**	**4.15**	**-**	**15.0**	**16.2**	**17.3**	**-**	**6.0**	**29.5**	**29.7**
Biomolecules	**1.92**	**2.91**	**3.79**	**4.35**	**-**	**11.3**	**17.2**	**19.4**	**-**	**7.6**	**26.4**	**30.7**
Control	**1.93**	**1.95**	**2.05**	**2.50**	**-**	**5.2**	**5.5**	**5.8**	**-**	**4.0**	**9.0**	**13.5**

Experiments were done as described in table 1

Table 4 Effect of various molecules on the growth of pea

Type of Molecules	**Type of Plant**											
	***Pisum sativum* L**											
	Wet-weight (g)				**Length (cm)**							
					Root				**Shoot**			
	Days											
	I	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**
Sodium nitrate	**2.80**	**3.90**	**4.35**	**5.55**	**-**	**3.0**	**8.0**	**8.2**	**-**	**8.0**	**9.2**	**9.5**
Urea	**2.56**	**3.50**	**3.85**	**4.05**	**-**	**2.6**	**8.5**	**8.7**	**-**	**1.9**	**8.0**	**8.2**
Ammonium Sulfate	**2.91**	**3.95**	**5.35**	**6.05**	**-**	**4.9**	**18.5**	**10.2**	**-**	**5.0**	**11.0**	**14.0**
Glycine	**2.94**	**4.05**	**4.69**	**5.90**	**-**	**3.5**	**16.2**	**16.8**	**-**	**6.2**	**7.2**	**7.5**
Biomolecules	**2.42**	**4.05**	**5.79**	**6.15**	**-**	**4.5**	**17.2**	**19.4**	**-**	**8.0**	**13.2**	**14.2**
Control	**2.56**	**3.75**	**4.15**	**5.05**	**-**	**1.5**	**7.0**	**7.2**	**-**	**3.8**	**8.3**	**8.5**

Experiments were conducted as described in table

It showed increase of total biomass and growth (fig. 1-4). However, in control sets, there was little increase of total biomass when cotton was the matrix for cultivation. It is different when soil was the matrix (data not shown) . It showed a little increase of total bio - mass which was less compare to the sets where fertilizers were used. Out of all these experimental sets the increase of growth in highly significant when bio-molecules were used as a fertilizer.

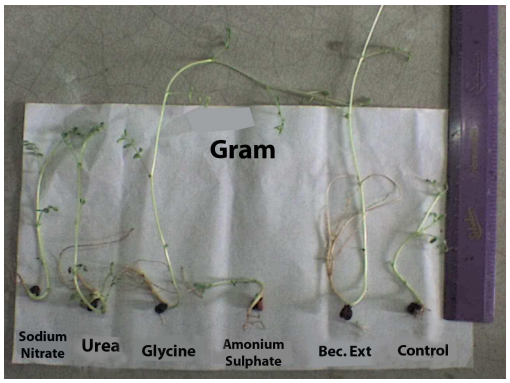

Fig.1 Growth of plantlets of gram in presence of various molecules.

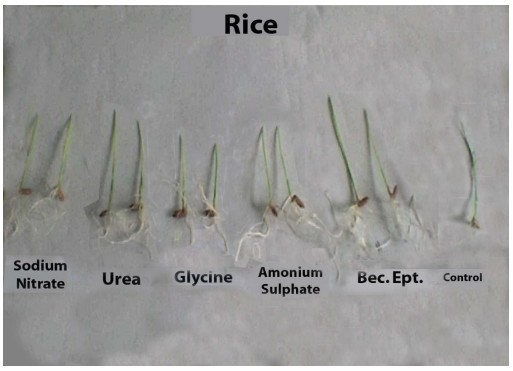

Fig. 2 Growth of plantlets of rice in presence of various molecules. Growth conditions as per fig.1

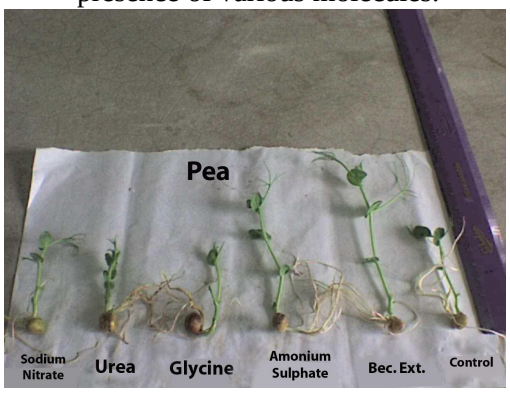

Fig. 3 Growth of plantlets of pea in the presence of various molecules. Growth conditions as per fig.1

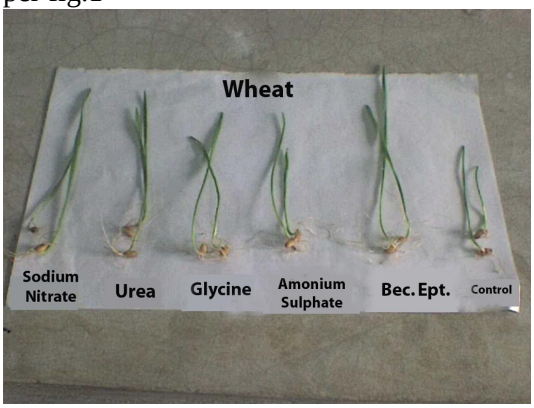

Fig. 4 Growth of plantlets of wheat in the presence of various molecules. Groth conditions as per fig.1

Perhaps it is due to the easy accessibility of these molecules to the experimental plants. Importantly, these molecules are similar type of bio-molecules, which are the makers of a plant body. Transportation of these molecules are also easy in a plant (Stuartchapin et al , 1998). Moreover, transport of nutrients in plant system is cascaded with plethora of cellular molecules that will recognize these molecules which are supplied as a fertilizer. (Allen ,1995). The advantages of this fertilizer are many over the conventional fertilizers because it supplies bio-molecules as well as water. It is liquid and easily accessible to the matrix of cultivation as well as to the plants. In the preparation process of these bio-molecules we have used lysozyme and proteinase K which are expensive and addition of these will increase the cost. Therefore, it is better to use mechanical process to make micro molecules from macromolecules of a

bacterial cell . Perhaps, sonication of bacterial cell will be the appropriate technique (Sen 2007) that can be used to prepare these molecules industrially. Importantly use of living bacterial cell or any kind of living organism compete for nutrition with crop plant in a cultivation land (Rao et al 1983). Mostly the bacterial cells remain as intact cell in the soil as bacterial cell wall is structurally very strong. Decomposition of bacterial cell may occur very slowly however it occurs only in non- sporulating bacteria. . However, rigorous field experimentations are highly desirable to test the novelty of addition of these molecules as bio-fertilizer.

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