

Original Research Article

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## Cyanobacterial Culture Acts as Good Organic Biostimulant on *Anethum graveolens*

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### ABSTRACT

Beneficial microbes can act as plant growth stimulants without affecting soil health thus in present study one of the beneficial photosynthetic microbe was used to study its effect on *Anethum graveolens* (Dill) plantlet. The *Anabaena* culture was used in defined concentration and its effect on germination rate, shoot length, root length, number of branches, pigments content, protein and nutrients quality of soil was carried out. Germination rate was found to be 40% more in seed treatment (ST) as compared to untreated (UT)/controlled Dill seeds. Shoot length was 10% higher in foliar spray treatment (FST) plants as compared to UT Dill plantlets. Numbers of branches were 13% more in FST treatment than UT. Similarly there was 2-6% increase in Chlorophyll a and Chlorophyll b pigments in FST and ST plantlets as compared to UT Dill plantlets. Also there was 14% increase in both organic carbon and nitrogen content of soil after FST treatment of Dill and 27% increase in both organic carbon and nitrogen content of soil after ST treatment of Dill. The results showed positive effect of seed and foliar spray treatment of cyanobacterial culture with respect to above mentioned parameters on Dill plantlets.

#### Keywords

Cyanobacteria, Dill,  
Foliar spray, Seed  
treatment,  
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### Introduction

Chemical fertilizers, if used in large quantity are harmful for soil health and beneficial microbes. Because of ignorance and less education farmers use more fertilizers with aim to produce large produce and thus spoil the soil health thus an eco-friendly alternative is required. Cyanobacteria are photosynthetic microbes which are primitive to plants. They

share similar mechanism of carbon dioxide fixation and its extracts are known for plant growth (Essa *et al.*, 2015; Swarnalakshmi *et al.*, 2013).

Cyanobacterial exudates contains gibberellins, linalool, n-acetyl-D-glucosamine, niacinamide and dihydroxyphenyl glycol which might be responsible for cell enlargement, cell division and root initiation, along with their role in the

activation of the antioxidative defense enzymes of plants (Essa *et al.*, 2015). According to Riahi *et al.*, 2013, Cyanobacteria benefit plants by producing amino acids, polypeptides, growth promoting regulators, vitamins, antibacterial and antifungal substances that improve plant growth and productivity. Cyanobacterial treatments are known to increased Barley and Fenugreek plants germination percentage, length of shoot, fresh and dry weights along with photosynthetic pigments (Chlorophylls and carotenes), proteins, glutamic-oxaloacetic and glutamic-pyruvic transaminases (Ismail and Abo-Hamad, 2017). They had also attributed the plant growth promoting effect to different bioactive materials like phytohormones, exopolysaccharides, nitrogen, phosphorus and potassium in the cyanobacterial biomass (Ismail and Abo-Hamad, 2017).

The genus *Anethum* is Greek word which means strong smelling (Jana and Shekhawat, 2010). It has enormous Ayurvedic medicinal value in promoting digestion, abdominal discomfort, colic, eye diseases, rheumatic, other swellings of joints and uterine pains (Khare, 2004, Jana and Shekhawat, 2010). It is also used in more than 56 ayurvedic preparations (Ravindran and Balachandran, 2005). It is believed to be the originated from South-west Asia or South-east Europe (Bailer *et al.*, 2001). Dill is known to give relief from Insomnia, respiratory disorders, Boosts immunity and bone health, maintains proper menstrual cycle, prevents cancer and microbial infection, helps in digestion and cures diarrhea and dysentery, reduces pain and inflammation caused by Arthritis and gout, etc (Choudhary *et al.*, 2015). It is very common vegetable used as food in India.

On the basis of biostimulants effect, the experiments were planned on *Dill* with respect to different parameters like germination rate, root length, shoot length, number of branches,

pigments, proteins, soils organic carbon, Nitrogen, Potassium and Phosphate to study its effects on these parameters in eco-friendly way.

## Materials and Methods

### Seeds, cyanobacterial strain and media composition

*Anethum graveolens* seeds were brought from the local farmers market of Yavatmal, Maharashtra and stored at room temperature till further use.

The Cyanobacterial culture (*Anabaena ambigua*) was procured from National Collection of Industrial Microorganism Pune (NCIM) and maintained on Fog's medium at light intensity of ~6000 lux at Room temperature.

### Composition of Fog's medium

MgSO <sub>4</sub> .7H <sub>2</sub> O	0.2 g
K <sub>2</sub> HPO <sub>4</sub>	0.2 g
CaCl <sub>2</sub> .H <sub>2</sub> O	0.1 g
*Micronutrient solution	1 ml
*Fe-EDTA solution	5.0 ml
Agar	10.0 g
pH	7.5
Distilled water	1.0 L

### Micronutrients solution

H <sub>3</sub> BO <sub>3</sub>	286.0 mg
MnCl <sub>2</sub> .4H <sub>2</sub> O	181.0 mg
ZnSO <sub>4</sub> .7H <sub>2</sub> O	22.0 mg
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	39.0 mg
CuSO <sub>4</sub> .5H <sub>2</sub> O	8.0 mg
Distilled water	100.0 ml

### Fe-EDTA solution

In hot distilled water (D/W), 745.0 mg of Na<sub>2</sub>EDTA was dissolve completely and then

added 557.0 mg of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ . The solution was boiled for few minutes and made up the volume to 100 ml by D/W.

### **Determination of *A. ambique* growth**

Optical density of *A. ambique* culture was measured at 750nm using spectrophotometer with Fog's culture media as blank.

### **Study of *A. ambique* culture on different parameters of Dill**

#### **Experimental design**

The treatment of Dill (seed and plant treatment) were performed into 3 sets, as

I) Untreated seeds (UT) / Control (5 set)  
- Seeds/plantlets without any *A. ambique* culture treatment.

II) Seed treatment (ST) – (5 set)  
- *A. ambique* culture of optical density 1 was used to coat the Dill seeds. The seeds were dipped in cyanobacterial culture for 30 mins and then air dried before sowing them into soil.

III) Foliar Spray treatment (FST) – (5 set)  
- *A. ambique* culture of optical density 1 was sprayed on plantlets by foliar spray application using spray pump.

### **Growth condition for treated and untreated dill**

The treated and untreated plantlets/vegetables were grown at light intensity of ~6000 lux at Room temperature.

### **Determination of rate of germination**

The percent rate of germination was calculated by dividing number of seeds sown and number of plantlets grown, multiplied by 100.

### **Determination of shoot length of plantlets**

The length of shoots was measured in cm

### **Determination of number of branches**

The number of branches were visually observed and noted.

### **Determination of root length**

Root length of plantlets was measured in cm.

### **Determination of pigments**

Chlorophyll and carotenoids content of plantlets were determined by using Rathod *et al.*, (2016). The leaves of all the plantlets from same set (FST, ST and UT) were taken together and values were determined.

### **Study of protein content**

Protein content of plantlets from all sets was determined by using Biuret method. The complete plantlets from same set (FST, ST and UT) were taken together to calculate total protein content. BSA was used as standard for making standard curve from Himedia.

### **Study of N, P, K and organic carbon content of soil**

N, P, K and organic content of soil before and after treatment of cyanobacterial culture as stimulant was analyzed at Krishi vighan Kendra of Yavatmal using standard protocols.

### **Results and Discussion**

The effect of *A. ambique* culture on different parameters of Dill was studied to understand its effect on Dill growth organically because of its enormous medicinal properties. Table 1 shows the effect of cyanobacterial seed treatment on germination rate.

Percent germination of seeds is one of the important factors in agriculture. Percent increase in germination rate of ST was found to be 40% more in ST as compared to control (UT) seeds which was found to be higher. Essa *et al.*, (2015) had also got 30% increase in the seed germination rate of Sorghum by *Anabaena oryzae* exudates. Similarly Kumar and Kaur, (2014), observed 62.9% increased in wheat germination by using filtrate of *Anabaena* species namely *Anabaena variabilis*. Thus seed treatment with *A. ambigua* culture can be an excellent strategy to increase the germination rate. It may help in stimulating germination process which needs to be study in detail.

After germination rate studies, effect of foliar treatment on grown plants was studied in another set of experiment (FST). The result of foliar spray treatment is given in Table 2.

FST plantlets showed 10 % increase in shoot length than UT which was significant. Similarly 84% increase in Cucumber and 56% increase in Tomato were observed by Shariatmadari *et al.*, (2013) by using *Anabaena* culture. Thus foliar application of cyanobacteria can be a good strategy to increase the shoot length of Dill and other vegetables or plants.

After studying the effect on shoot length, effect on branching was found out after foliar spray treatment. Table 3 shows the results of foliar spray on Dill.

Number of branches in FST was found to be 12.57% more than UT. Similar studies were carried out by us in *Coriandrum* were percent increase in branching was found to be 22% (Data not shown). We can conclude that spraying has positive effect on branching as well and thus can increase the yield of vegetable or crop significantly.

Studies with respect to root length were carried out but it was found to be similar in FST, ST and UT of Dill.

The effect of seed treatment and foliar spray treatment on pigments was studied on Dill, the results are as mentioned in Table 4 below.

The parameters such as total chlorophyll was found to be 3.45% higher in FST and 1.39% higher in seed treatment as compared to controlled plantlets grown in same conditions. Chlorophyll a was found to be 5.53% more in FST and 2.29% more in ST plantlets as compared to control UT plantlets. Ismail and Abo-Hamad, (2017), also found increase in Chlorophyll a and Chlorophyll b in Barley and Fenugreek using *Anabaena* culture. The carotenoids were found to be slightly increased i.e. 2.34% in FST and 1% in ST plantlets as compared to UT plantlets of Dill. It showed that carotenoids did not change to higher level in case of Dill.

The effect of *Anabaena* culture on Dill plantlets on total protein content was studied and shown in Table 5.

No difference was found in FST and UT plants as compared to controlled UT plantlets. Ismail and Abo-Hamad, (2017) got increase in protein content who studied the effect of *Anabaena* culture on Barley and Fenugreek. It might be because of inability of dill leaf to absorb the amino acids present in intact *Anabaena* culture as opposed to Ismail and Abo-Hamad, (2017), who utilized the cyanobacterial extract. The effect of foliar spray and ST on soil nutritional content was also studied to understand its impact on soil. Both the treatments showed positive impact on soil where organic carbon content and nitrogen content was found to be 14% higher in FST and 26.78% higher in ST plantlets respectively as compared to UT Dill plantlets (Table 6).

**Table.1** Comparison of ST and UT with respect to germination rate of Dill (number of seeds germinated)

SET	ST	UT
1 <sup>st</sup>	3	3
2 <sup>nd</sup>	2	1
3 <sup>rd</sup>	3	2
4 <sup>th</sup>	3	2
5 <sup>th</sup>	3	2
<b>AVERAGE±SD</b>	2.8±0.40	2±0.63

The t-test value of ST was significant at  $p < 0.06$

**Table.2** Comparison of FST and UT plantlet with respect to Shoot length

SET	FST(cm)	UT(cm)
1 <sup>st</sup>	10.47	10.83
2 <sup>nd</sup>	11.66	9.85
3 <sup>rd</sup>	11.15	9.70
4 <sup>th</sup>	10.48	9.20
5 <sup>th</sup>	10.60	9.85
<b>AVERAGE±SD</b>	10.87±0.47	9.88±0.53

The T-test value of FST was significant at  $p < 0.02$

**Table.3** Comparison of FST and UT plantlets with respect to Branching of Dill (number of branches)

SET	FST	UT
1 <sup>st</sup>	7	4.66
2 <sup>nd</sup>	7.33	4.5
3 <sup>rd</sup>	6	4.5
4 <sup>th</sup>	6	7.5
5 <sup>th</sup>	6.5	8
<b>AVERAGE±SD</b>	6.57±0.53	5.83±1.57

**Table.4** Total Chl, Chl-a, Chl-b and Carotenoid Contents of different treatments in Dill plantlets ( $\mu\text{g}\cdot\text{gm}^{-1}$ )

Parameters	FST	ST	UT
<b>Total Chlorophyll</b>	202.94	198.91	196.17
<b>Chlorophyll- a</b>	72.38	70.16	68.59
<b>Chlorophyll- b</b>	130.63	128.82	127.64
<b>Carotenoid</b>	5.34	5.28	5.47

**Table.5** Total Protein Content of FST, ST and UT plantlets (mg.gm<sup>-1</sup>)

	FST	ST	UT
Protein content	85.22	84.77	85.10

**Table.6** Effect of *Anabaena* culture on nutritional quality of soil

SET	Organic carbon(%)	N (kg.ha <sup>-1</sup> )	P (kg.ha <sup>-1</sup> )	K (kg.ha <sup>-1</sup> )
FST	0.64	286.08	22.2	724.8
ST	0.71	317.37	24.0	503.0
UT	0.56	250.32	22.7	760.5

The phosphate content was almost similar with slight increased in seed treated plants as compared to UT plantlets soil. The potassium content was found to be decreased in both the treatment which might be because of increase in Organic carbon and nitrogen content of soil which require more potassium from the soil for the plant growth, which were evident from earlier studies.

In conclusion, the *Anabaena* culture is able to increase the germination rate in Dill after seed treatment which can be used for increased germination rate. The Foliar treatment can positively affect the shoot length and number of branching which may help in increasing weight of the Dill plant. The pigments such as Chlorophyll a and Chlorophyll b can be increased which are associated with increased photosynthetic rate after *Anabaena* treatment. Also the organic content and nitrogen content of soil may get increased because of cyanobacterial application. Intact culture of *Anabaena* may not have positive response on protein content. Overall Cyanobacteria can positively affect the Dill plantlet after seed or foliar spray treatment.

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