

Original Research Article

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Study of *Anabaena ambigua* on Growth Parameters of *Coriandrum sativum* after Seed and Foliar Spray Treatment

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ABSTRACT

The present study aimed at studying the biostimulant effect of cyanobacterial cultures on the growth parameters of *Coriandrum Sativum*. Cyanobacteria helped in absorption of atmospheric carbon dioxide and reduced its concentration to the level which is suitable for evolution of higher organisms. It is one of the oldest microbes and has evolved in many beneficial ways. It is known to produce exopolysaccharides, phytohormones and also have inherent nitrogen, phosphate and potassium for healthy growth of other plants. In this study, intact cyanobacterial cells were used for seed treatment and as foliar application on *Coriandrum sativum* and its effects were compared to untreated plants with respect to different growth parameters. In case of seed treated (ST) plants the percent germination, number of branches, root length, Chlorophyll a, Chlorophyll b were found to be increased as compared to untreated (UT) plants. While in case of foliar spray treatment (FST), shoot length, number of branches, Chlorophyll a and Chlorophyll b content were found to be increased as compared to UT plants. Thus application of cyanobacterial culture on vegetables may result in increased production organically. The results showed positive effect of both seed treatment and foliar spray treatment on *C. sativum* plants.

Keywords

Anabaena, *Coriandrum*,
Biostimulant, Foliar,
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Introduction

Coriandrum Sativum (Coriander) also called as Kothimbir, Dhaniya, Harapatta, etc is widely used vegetable to make different food recipes because of its taste, aroma and nutritional contents (Hammer and Heller, 1998). Along with that it has several health benefits as it is rich in Vitamin (Hwang *et al.*, 2014). Coriander helps in skin related disorder, reducing cough, reducing blood

pressure, reducing Diarrhea, reducing the Cholesterol levels in the bloods (Hwang *et al.*, 2014).

Farmers grow Coriander throughout the year because of its high demand in the market and taste. They use different chemical fertilizers for its growth which is deteriorating the health of soil. An alternative to chemical fertilizers, cyanobacterial strain was used as biostimulant to study its effect on its growth, productivity

and nutritional content of this vegetable (Swarnalakshmi *et al.*, 2013).

GC-MS analysis of *Anabaena* exudates showed the presence of n-acetyl-D-glucosamine, gibberellins, linalool, niacinamide and dihydroxyphenyl glycol (Essa *et al.*, 2015). *Anabaena* culture contains these bioactive compounds 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). Cyanobacteria have been reported to benefit plants by producing amino acids, polypeptides, growth promoting regulators, vitamins, antibacterial and antifungal substances that improve plant growth and productivity (Riahi, 2013). Ismail and Abo-Hamad (2017) studied the effect of *Anabaena* culture on growth parameters and physiological aspects of Barley and Fenugreek.

Cyanobacterial treatments increased those plants germination percentage, length of shoot, fresh and dry weights. The photosynthetic pigments (Chlorophylls and carotenes), proteins, glutamic-oxaloacetic and glutamic-pyruvic transaminases activities were also increased. They had attributed the plant growth promotive effect to the bioactive materials like exopolysaccharides, phytohormones, nitrogen, phosphorus and potassium in the cyanobacterial biomass (Ismail and Abo-Hamad 2017).

On the similar lines the experiments were planned on *C. Sativum* and analysis with respect to germination rate, root length, shoot length, number of branches, chlorophyll pigment, proteins, soils organic carbon, Nitrogen, Potassium and Phosphate were carried out in two different sets of experiments (Seed treatment and Foliar spray treatment compared with untreated plants).

Materials and Methods

For the current experiment, *Coriandrum Sativum* seeds were brought from the local farmers market of Yavatmal and stored at room temperature.

Cyanobacterial strain and media composition

The Cyanobacterial culture (*Anabaena ambigua*) was ordered 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 ₄ .7H ₂ O	0.2 g
K ₂ HPO ₄	0.2 g
CaCl ₂ .H ₂ O	0.1 g
*Micronutrient solution	1 ml
*Fe-EDTA solution	5.0 ml
Agar(Difco)	10.0 g
pH	7.5
Distilled water	1.0 L
*Micronutrients solution	
H ₃ BO ₃	286.0 mg
MnCl ₂ .4H ₂ O	181.0 mg
ZnSO ₄ .7H ₂ O	22.0 mg
Na ₂ MoO ₄ .2H ₂ O	39.0 mg
CuSO ₄ .5H ₂ O	8.0 mg
Distilled water	100.0 ml
*Fe-EDTA solution	

In hot distilled water (D/W), 745.0 mg of Na₂EDTA was dissolve completely and then added 557.0 mg of FeSO₄.7H₂O.

The solution was boiled for few minutes and made up the volume to 100 ml by D/W.

Study of effect of *A. ambigua* culture on different parameters of coriander germination and growth

Determination of growth of *A. ambigua*

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

Experimental design

The treatment of Coriander (seed and plant treatment) were divided into 3 sets, as

Untreated seeds (UT) / Control 5 set

Seeds/plantlets without any *A. ambigua* culture treatment.

Foliar Spray treatment (FST) – 5 set

A. ambigua culture of optical density 1 was sprayed on plantlets by foliar spray application using spray pump.

Seed treatment (ST) – 5 set

A. ambigua culture of optical density 1 was used to coat the Coriander seeds.

The seeds were dipped in cyanobacterial culture for 30 mins and then air dried before sowing them into soil.

Growth condition

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

Parameters determined

The plantlets were studied for various parameters mentioned underneath after seed treatment and foliar spray treatment with *A. ambigua* culture.

Study of seed rate germination

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

Study of shoot length of plantlets

The length of shoots was measured in cm using plastic scale.

Study of number of branching of plantlets

The number of branches were visually observed and noted.

Study of root length of plantlets

Root length of plantlets was measured using plastic scale in cm.

Analysis of N, P, K and organic carbon content of soil

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

Study of Chlorophyll and Carotenoids content

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

Study of protein content

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

Results and Discussion

Average germination rate of Coriander for each set of ST and UT was taken and the results were shown in Table 1.

Percent increase in germination rate of ST was found to be 28.57% more as compared to UT. The results indicated positive effect of *Anabaena* seed treatment on Coriander plantlet. Similar results were observed by Essa *et al.*, (2015), where he had got 30% increase in the seed germination rate of Sorghum by *Anabaena oryzae* exudates. Also Kumar and Kaur, (2014), observed 62.9% increased in wheat germination by using another *Anabaena* species namely *Anabaena variabilis* filtrate. Thus seed treatment with *A. ambigua* culture can be used as germination rate increasing strategy for different vegetable and crops.

Effect of *Anabaena* on shoot length of coriander was studied and given in Table 2.

Percent increase in shoot length of FST plants was found to be more than UT plants. Percent increase in FST was 6.28% as compared to UT plantlets. In case of ST plants, plant height was less than UT by 4.02%, it means foliar

application is essential for plant's further growth and seed treated plants could help in increasing percent germination. Shariatmadari *et al.*, (2013) also observed 42% increase in plant height of Squash plant, 84% increase in Cucumber and 56% increase in Tomato by using *Anabaena* culture. The positive effect of cyanobacterial culture on shoot length or plant height was evident from all these studies.

To study the effect of *Anabaena* on branching of coriander so as to increase the weight of this vegetable, experiment was carried and observations were documented as shown in Table 3.

The percent increase in branching of ST was more as compared to UT plants. The percent increase in ST was found to be 36.84% more than UT. For FST it was found to be 21.92% more than UT plant. T-test value of ST was found to be significant. It clearly indicated the role of cyanobacterial culture on increase in branching of coriander plantlet which was beneficial for plant growth.

The effect of *Anabaena* on root length was observed and mentioned below in Table 4.

Table.1 Effect of *Anabaena* on germination rate of Coriander (number of seeds germinated)

Set	ST	UT
1 st	2	1
2 nd	2	1
3 rd	2	1
4 th	2	2
5 th	1	2
AVERAGE±SD	1.8±0.4	1.4±0.48

Table.2 Effect of Anabaena on shoot length of coriander (cm)

SET	FST	ST	UT
1 st	8.33	4.8	6
2 nd	6.61	5.95	7
3 rd	5.85	6	5.5
4 th	5.2	7.05	5.3
5 th	7	6	7.25
AVERAGE±SD	6.6±1.066	5.96±0.712	6.21±0.785

Table.3 Effect of Anabaena on branching of coriander plantlet (number of branches)

SET	FST	ST	UT
1 st	5	5	4
2 nd	2	6	2
3 rd	5.5	6	4
4 th	6	5	5
5 th	4.66	4	4
AVERAGE±SD	4.63±1.39	5.2±0.74	3.8±0.97

Table.4 Effect of Anabaena on root length of coriander (cm)

SET	FST	ST	UT
1 st	1.6	2.6	3.2
2 nd	2.1	4	2.6
3 rd	2	2.6	1.9
4 th	2	2.6	1.9
5 th	-	-	-
AVERAGE±SD	1.92±0.192	2.95±0.606	2.4±0.543

Table.5 Total Chl, Chl-a, Chl-b, Carotenoid Content of Coriander (µg/gm) in different FST, ST and UT plantlets

Parameters	FST	ST	UT
Total Chlorophyll	194.724	198.024	188.435
Chlorophyll- a	67.6914	70.143	65.362
Chlorophyll- b	127.102	127.951	123.140
Carotenoid	5.367	5.460	5.421

Table.6 Total Protein Content of FST and ST as compared to UT plantlets (mg/gm)

	FST	ST	UT
Total Protein	86.207	86.427	85.217

Table.7 Effect of *Anabaena* on nutritional quality of soil

	Organic carbon (%)	N (kg/ha)	P (kg/ha)	K(kg/ha)
FST	0.44	196.68	30.1	255.0
ST	0.40	178.8	28.2	270.0
UT	0.35	156.45	29.1	280.0

The percent increase in ST was more i.e. 22.91% as compared to UT plants. Contrary, percent decrease in FST was 19.79% less as compared to UT. Similarly Shariatmadari *et al.*, (2013), found improvement in rooting of Squash, Cucumber and Tomato plants after using *Anabaena* culture. This indicated the positive effect of seed coating of *Anabaena* culture on root length whereas negative effect after foliar spray on rooting. Although the root length was small but the shoot length was highest in foliar application, which means foliar application increases the shoot length by acting on it and seed treatment stimulates the rooting. It also means plants were absorbing nutrients via leaf and there might not have much requirement of long root formation in foliar application. In future research both the effects together (seed treatment along with foliar application) will be studied.

The effect of foliar treatment on pigments such as total chlorophyll (Chl), Chlorophyll-a, chlorophyll-b and carotenoids were studied and mentioned below in Table 5.

The total chlorophyll which determines the indirect overall photosynthetic capabilities was found to be slightly higher in FST and ST plantlets as compared to control UT plantlets. It was found to be 3.33% and 5% more in FST and ST with respect to UT plantlets. Chlorophyll-a was found to be 3.6% and 7.3% more in FST and ST compared to UT plantlets. Chlorophyll-b was also slightly got increased by 3.2% and 3.9% in FST and ST compared to UT plantlets. Similar results with increase in Chlorophyll a and Chlorophyll b

were observed by Ismail and Abo-Hamad, (2017) who studied the effect of *Anabaena* culture on growth parameters and physiological aspects of Barley and Fenugreek. There was no major difference found in carotenoids of FST and ST as compared to UT plantlets. Plants usually produce carotenoids in stressed conditions of high light and temperature (Rathod *et al.*, 2016). The reason might be because the plantlets were grown in controlled conditions of light intensity. It will be interesting to see the effect under open atmospheric condition.

It was interesting to study the effect of *Anabaena* culture on coriander to determine the total increase in protein content of plantlets. It was found that there was not much difference found in FST and UT plants compared to controlled UT plantlets (Table 6). It was contrary to the results where increase in protein content was observed by Ismail and Abo-Hamad (2017) who studied the effect of *Anabaena* culture on growth parameters and physiological aspects of Barley and Fenugreek. It means that the proteins or amino acids present in *Anabaena* culture were not been able to directly utilize by the leaves in our case. Also, Ismail and Abo-Hamad (2017), utilized the cyanobacterial extract and not the complete cells and that can be one of the reasons of not increase in protein content in our case.

After seed and foliar treatment organic carbon, nitrogen, phosphorus and potassium content of soil were determined to study its effect. It was found that *Anabaena* culture positively increased the organic carbon

content by 26% in case of FST treatment as compared to control Table 7. Also 14% increase in ST was found as compared to control.

Thus *Anabaena* application is beneficial for increasing carbon content of soil. Similarly nitrogen content was found to be increased in FST and ST by 25% and 14% as compared to UT, which was positive sign. Although the plantlets did not directly take up the proteins or amino acids as shown earlier but it definitely increased the soil nutrients content which may be beneficial for subsequent vegetables or crops. The phosphate content of soil was slightly increased in case of FST treatment while it was slightly decreased in case of ST as compared to UT. The potassium content was found to be reduced in case of FST and ST treatment by 9.8% and 3.7%, it might be because as other components (organic carbon and nitrogen) were available, potassium had got utilize in the process. Overall *Anabaena* positively impacted the soil quality and it could be one of the better options for organic farming.

The cyanobacterium is able to induce and increase seed germination in Coriander plants if applied directly to the seeds. The grown plants also show increase in branching, root induction and increase root length. The photosynthetic pigments like chlorophylls also get positively influenced by plants grown after seed treatment.

Foliar spray is very effective in increasing the shoot length, branching along with Chlorophyll a and Chlorophyll b in case of Coriander plants. Also the organic content and nitrogen content can be get increased by these treatments. Thus both the treatments showed positive effect on growth of plants and can be used as biostimulant for increased productivity and nutritional quality of vegetable plants.

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