



## Research Article

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# Effect of Coir Pith Based Cyanobacterial Biofertilizer for Improving Fatty Acid Contents in *Arachis hypogaea* L (Groundnut) Oil Seeds

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## Abstract

Natural and organic farming techniques have the lowest expenditure as compared to inorganic fertilizers, and are harmless to human, animals and environment. In the present work, a field experiment was carried out to evaluate the effect of using chemical fertilizer and coir pith based cyanobacterial biofertilizer (cyanopith and cyanospray) for improving the quantity and quality of fatty acid contents in oil seeds of *Arachis hypogaea* L. (Groundnut). It was cultivated in a field treated with 200 kg/acre cyanopith and 0.3% cyanospray. After treating this plant with chemical and cyanobacteria biofertilizers, fatty acid compositions in the seeds have been analyzed by Gas chromatograph. The results of this study showed that cyanopith treated seeds have accumulated more fatty acid methyl esters in which a higher concentration of omega-3 fatty acids methyl esters like eicosapentaenoic acid (C20:5n3) and docosahexaenoic acid (C22:6n3) found in the oil seeds. It was comparatively lesser than that was obtained in it by using chemical fertilizers. Thus, it concluded that improved quality of fatty acid contents in groundnut seed can possibly be achieved by cyanobacterial biofertilizer, suggesting of using it as agronomic fertilizers in the field.

**Keywords:** Cyanobacterial biofertilizer; *Arachis hypogaea* L; Fatty acid Methyl Esters; Coir pith; Food quality; Cyanospray; Fatty acids

## Introduction

Groundnut (*Arachis hypogaea* L.) is an annual oil seeds crop in India. It grows in tropical and subtropical regions. Groundnut has plenty of oil, protein, biotin especially niacin<sup>1,2</sup>. Nutritional variation and quality in oil seeds may depend on variety, climate, soil, nature, harvesting practices and pest management methods<sup>3</sup>. Vegetable oil obtained from groundnut has saturated and

unsaturated fatty acids which are used in the preparation of human food, animal and poultry feed. The quality of the fatty acid contents in groundnut oil seeds is one of the major concerns in human health, however, it can be improved by different approaches, but these are cost effective and time consuming strategies<sup>4-7</sup>.

Cyanobacteria are the largest and most widely distributed species on earth. They are oxygen releasing gram negative photosynthetic organisms commonly grown on aquatic and

terrestrial habitats. They are filamentous or unicellular grown like mats on surface of bare soil as primary colonizers<sup>8,9</sup>. Many cyanobacteria can able to fix atmosphere nitrogen into available nitrogen for the growth of plants. The nitrogen fixing aquatic fern *Azolla pinnata* maintains a symbiotic relationship with cyanobacteria *Anabaena azolla* inside the leaf cavities. Accordingly, cyanobacteria can be used as biofertilizer for better crop productio<sup>8-11</sup>.

Coconut (*Cocos nucifera* L.) is one of the most useful extensively cultivated palms in tropical countries like India. The coir dust and coir pith are major waste products of coir yarn industries. As coir pith has high lignin content and has fibrous matter, it takes decades to decompose causing environmental pollutions and disposal problems. Cyanobacteria are found to degrade coir pith at ambient conditions, and the degraded products consisting cyanobacterial biomass and coir pith by products are served as biofertilizer (cyanopith plus cyanospray) to enhance the plant growth and yield as reported earlier<sup>9-11</sup>. Since, a coir pith degraded products with cyanobacteria is known as cyanopith biofertilizer. Therefore, the present work was aimed to evaluate the effect of coir pith based cyanobacterial biofertilizer (cyanopith and cyanospray) on improving the quality of fatty acid contents in groundnut oil seeds in the field.

## Experimental

### Growth and maintenance of cyanobacteria

A fresh water cyanobacterium, *Oscillatoria annae*, was procured from Germplasm stock of National Facility for Marine Cyanobacteria (NFMC), Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. The culture was maintained in BG11 medium<sup>12</sup> at 1500 lux at 25 ± 2°C with 10/12 hours light and dark cycle.

### Field experimental conditions

Coir pith was collected locally from a coir yarn industry. The culture of *O. annae* was inoculated into coir pith in 1:10 ratio (wet: dry weight) for mass cultivation in a pit. After 20-25 days of incubation, the whole content was separated into pellet and supernatant. The dried pellet (cyanopith) was used as a basal fertilizer and the supernatant (cyanospray) was used as foliar spray for cultivation of *A. hypogaea* L. The experiment was carried out on cultivation of *A. hypogaea* L. treated with concentration of 200 kg/acre cyanopith

and 0.3% cyanospray. After harvesting, the seeds were taken for analyzing fatty acids methyl esters (FAMEs) by gas chromatograph (Model Perkin Elmer Clarus 500 with capillary column) and the results were compared with control and chemical treated seeds.

### Extraction of Fatty Acid Methyl Esters

Groundnut (5g) seeds were taken from each treatment. Lipid extraction was done with chloroform:methanol mixture. A screw capped tube containing 200µl lipid and one ml saponification reagent prepared by dissolved 45g NaOH in methanol-water mixture (1:1) was kept at boiling temperature for 30 min. The 2 ml of methylation reagent (a mixture of 325 ml of 6NHCl and 275 ml methanol) was added to this mixture and then incubated the tubes at 80°C for 20 min. After that, 1.25 ml of extraction solvent (a mixture of 200 ml hexane and 200 ml of anhydrous diethyl ether) was added and the tube was rotated end-over-end for 10 min. The lower aqueous phase was discarded and the organic upper phase containing the FAMEs was transferred into a vial and kept in cold condition because the fatty acid methyl esters have lower boiling point and are more volatile than fatty acids.

### Estimation of FAMEs by Gas Chromatograph

The FAMEs were transferred to vials and 8 µl of the sample was injected into Elite wax (Cross bond – PEG) capillary column (30 m length, 250 µm diameter) through the injection port of the Gas chromatograph equipped with FID (Flame Ionization detector). Nitrogen was used as a carrier gas while hydrogen was used as a fuel gas at flow rate of 50 ml/min and Zero Air 450 ml/min. The temperatures of column, injection port and detector were 40°C to 260°C (increment of temperature), 250°C and 260°C, respectively. The FAMEs in the sample were measured from the retention time obtained for standard FAMEs according to Miller and Berger<sup>13</sup>.

## Results and Discussion

Gas chromatograph analysis showed that FAMEs capric acid methyl ester (C10), undecanoic acid methyl ester (C11), tridecanoic acid methyl esters (C13) and myristic acid methyl ester (C14) found in the oil seeds of groundnut in the field treated with cyanopith, but these FAMEs were absent in control and chemical treated seeds (Table 1). Oil seeds obtained from control plant have 6

numbers of saturated FAMES and 7 numbers of poly-unsaturated FAMES. Groundnut oil seeds obtained after the treatment with chemical fertilizers had 5 numbers of saturated FAMES and 7 numbers of poly-unsaturated FAMES. Although, we found 9 numbers of saturated FAMES and 8 numbers of poly-unsaturated FAMES in oil seeds produced as a result of using cyanopith. Moreover, omega-3 fatty acids contents such as eicosapentaenoic acid methyl esters (C20:5n3) and docosahexaenoic acid methyl esters (C22:6n3) were detected in oil seeds obtained from cyanopith treatment, which was higher than in control and chemicals treated seeds.

Both omega-3 and omega-6 fatty acids are essential poly-unsaturated fatty acids that cannot be synthesis in human body<sup>14</sup>. The fatty acids eicosapentaenoic acid and docosahexanoic acid are synthesized from alpha linolenic acid through desaturation and elongation processes<sup>15</sup>. This pathway is an important source of these long chain n-3 poly-unsaturated fatty acids in strict vegetarians who do not eat fish, but they can obtain poly-unsaturated fatty acids from variety of food products<sup>16</sup>. Thus the presence of such fatty acids in groundnut oil seeds obtained from cyanopith treatment may be useful to human body as an essential diet.

The variation in fatty acids composition and quality may be due to the treatment of biofertilizers such as cyanopith and cyanospray which would have enhanced the synthesis of essential fatty acids by unknown mechanism. The endosymbiotic *Anabaena* with *Azolla* leaf Type I and Type II consist 17 numbers and 13 numbers of FAMES and sporocarp Type I and sporocarp Type II contain 17 numbers and 13 numbers of fatty acids. Sporocarp Type I had more than twice quantity of cis-linoleic acid (C18:2) compared to leaf of *Anabaena* with *Azolla* as a consequence of differential gene expression in cyanobacteria<sup>8</sup>. The cyanobacteria produce many bioactive compounds including the phyto regulators that could be used in the *in vitro* production of vegetables, fruits and ornamental flowers<sup>17</sup>. Some cyanobacterial strains reported to produce 3-indole acetic acids, auxin, and cytokinin<sup>5,18</sup>. Moreover, carbohydrates and amino acids increased in the seedlings of *Helianthus annuus* L. when grown in effluent blended with cyanobacteria<sup>4</sup>. Coir pith based cyanobacterial biofertilizer and coirpith based vermicompost have already been reported to improve the growth and yield of rice and some medicinal plants, respectively<sup>7,11</sup>. Accordingly, it proved that coir pith based cyanobacterial biofertilizers would increase the nutrient value, particularly fatty acid contents,

**Table 1.** Composition of fatty acid methyl esters (g/100g seed) in oil seeds of *Arachis hypogaea* L.

FAMES	Carbon	Control	NPK treatment	Cyanopith treatment
<b>Saturated fatty acids</b>				
Capric acid methyl ester	C:10	-	-	0.12
Undecanoic acid methylester	C:11	-	-	1.38
Lauric acid methyl ester	C:12	1.04	0.32	0.91
Tridecanoic acid methyl ester	C:13	-	-	0.38
Myristic acid methyl ester	C:14	-	-	0.24
Palmitic acid methyl ester	C:16	3.03	4.49	1.61
Heptadecanoic acid methyl ester	C:17	9.66	8.09	4.71
Behenic acid methyl ester	C:22	5.74	0.17	2.66
Thicosanoic acid methyl ester	C:23	0.80	-	-
Lignoceric acid methyl ester	C:24	15.8	0.50	7.52
<b>Unsaturated fatty acids</b>				
Palmitoleic acid methyl ester	C16:1	-	-	0.15
Heptadecanoic acid methyl ester	C17:1n9	7.54	21.22	3.24
Oleic acid methyl ester	C18:1	0.32	1.53	0.17
Linolenic acid methyl ester	C18:3n3	1.07	3.87	0.29
Arachidonic acid methyl ester	C20:4n6	1.08	1.33	1.83
Eicosapentaenoic acid methyl ester	C20:5n3	0.93	0.18	1.93
Nervonic acid methyl ester	C24:1	0.56	0.15	1.13
Docosahexaenoic acid methyl ester	C22:6n3	0.26	0.16	2.49

in oil seed of *A. hypogaea* L. as compared to control and chemical treated seeds.

The *Oscillatoria annae* extract at concentration of 0.01% contains the auxin which regulates the growth parameters and yields of *Oryza sativa* L., *Hibiscus esculentus* L. and *Helianthus annuus* L.<sup>6</sup>. The *O. annae* foliar spray has the properties of plant growth promoting substance such as auxin, gibberellins and naphthalene acetic acid<sup>9</sup>. Fatty acids oleic and linoleic acids were found in the oil sample of groundnut seeds<sup>2</sup>. The major fatty acids in groundnut seed detected were palmitic, stearic, oleic, linoleic, arachidic and behenic acid and 67 to 87% total unsaturated fatty acids<sup>1</sup>. Earlier reports showed that the coir pith based cyanobacterial biofertilizer as basal and foliar spray promotes the growth of *Basella rubra* L. wherein better height, stem circumference, numbers of branches, numbers of leaves, numbers of flowers have been resulted<sup>10</sup>.

Overall, we concluded that a combined effect of cyanopith and cyanospray biofertilizer gave the best results due to the effects of enhanced nutrients uptake on growth of groundnut plants. The organic amendment also increased the quality seed production of the crop, with the highest seed yield and quality. Therefore, coir pith based cyanobacterial biofertilizer (cyanopith and cyanospray) would be helpful for better crop productivity and improved fatty acid contents with a high food quality.

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