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Research Article

ANALYSES OF BIOACCUMULATION OF ASTAXANTHIN IN FENUGREEK SPROUTS

Bharathi Ravikrishnan[1]*., Abirami Selvaraj[2]., Ragunathan Manickavallii Gurunadhan[2] and Jayanthi Jayaprakash[3]

[1]Department of Biotechnology, Guru Nanak College, Chennai-42
[2]Department of Advanced Zoology and Biotechnology, Guru Nanak College, Chennai-42
[3]G.S.Gill research Institute, Guru Nanak College, Chennai-42

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A B S T R A C T

In the present study an attempt was made to use the prawn exoskeleton as biofertilizer to grown fenugreek sprouts. The presences of astaxanthin from the prawn shell and in the astaxanthin treated group of plants were analysed by TLC. The antioxidant analyses of the control fenugreek sprouts and the astaxanthin treated groups showed that in the astaxanthin treated group the antioxidant level was found to increase from 56% to 76%. In spite of the good growth the total chlorophyll was found to decrease in the astaxanthin treated plant.

INTRODUCTION

Malnutrition status in India

Malnutrition is a multidimensional phenomenon and one of the most depressing issues in India. In broad terms, it may be divided into protein energy malnutrition and micronutrient deficiency. India has a high prevalence of micronutrient deficiency-related health risks, which can be improved by food fortification (Pam *et al*., 2014). According to 2015, February UNICEF report, India has the largest number of underweight children and the highest number of undernourished population. The only right solution for malnutrition and also eradicating hunger is to increase the crop productivity fortified with nutrients (Allen, 2006). Micronutrient deficiency results from inadequate levels of iron, folate, iodine, and various vitamins; including A, B_6, D, and E, in the body, leading various metabolic disorders. After the green revolution, definitely in India the food production has increased tremendously, but, there is a huge lacuna to fulfill the nutritional status (Arvind, 2013). Thus, there is an increasing demand for fortifying food to meet the nutrition requirements is vital.

Biological significance of Fenugreek plants

In many parts of world plant are used as the best source of medicine for many centuries (Nasroallah *et al*., 2013). Among them fenugreek is one of the oldest plant which is commonly used in traditional medicine in many parts of Asia (Nathiya *et al*., 2012). The whole plant of fenugreek especially at the sapling stage and also its seeds are known for their curative properties (Mullaicharam *et al*., 2013). The medicinal properties are associated with the antioxidant properties of fenugreek (Petropoulos, 2002). Fenugreek is commonly consumed in raw form or in form of cooked form or as dry form in order to cure obesity, cancer, microbial infection and also known to control diabetics (Hajimehdipoor *et al*., 2010). It is also known to wade of insects (Qureshi *et al*., 2005).

Prawn shell as biofertilizer

When crustacean shell wastes are left to decay on the sea shore it putrefies and becomes a useless alkaline compound (Prabu and Natarajan, 2012). But instead, crustacean exoskeleton waste can be used to develop a wide range of value added products. According to Thirunavukkarasu and Shanmugam (2009), the crustacean processing industries throughout world generated 60,000 tonnes of waste every year. According to Jeyanthi *et al*., 2012; when the seeds of tomato, gram and peas were found to germinate faster in the presences of prawn shell powder and the shoot and root length were found to increase considerably (Jeyanthi *et al*., 2015).

Corresponding author:* **Bharathi Ravikrishnan
Department of Biotechnology, Guru Nanak College,
Chennai-42

METHODOLOGY

Extraction and Characterization of Astaxanthin

The astaxanthin extracted from *Fenneropenaeus merguiensis* exoskeleton shell waste was extracted and characterized by TLC and UV-spectrum as reported by Abirami *et al*., 2015. The astaxanthin which was extracted by using acetone as solvent was subjected to evaporation and the extract was suspended in DMSO for the plant treatment.

The pigment was characterised by UV Spectroscopy. The spectrum scan was performed from 470 to 485nm and the peak was obtained at 480 nm, which confirms the presence of carotenoid. The concentration of the pigment was calculated by using the formula given below (Uma Nath *et al*.,2012).

$$AST\ (\mu g.g^{-1}) = \frac{AxDx10^{6}}{100xGx\ dxE^{1\%\text{-}1cm}}$$

Where: AST is concentration of astaxanthin, A is Absorbance, D is volume of the hexane extract (2ml), G is the weight of the sample in gms.→3 gms., d is width= 1 cm., E = 2100

Plant Treatment

The treatment of fenugreek sprouts with natural bioaccumulation of astaxanthin in the sprouts.

Effect of astaxanthin over seed germination

The seeds of fenugreek (methi) were soaked overnight. The soaked seeds were then tied in small bag of cotton cloth and were allowed to germinate and one bundle of seeds were maintained as control sample. During the germination process, the seeds expect the control ones were treated with 100µl of DSMO for four days.

Effects of Astaxanthin over sprouting

The fenugreek seeds were sowed and were allowed to germinate under standard condition. The paper cups were divided into two batches containing eight paper cups (Ref.Figure.1). After sprouting the 100µl astaxanthin was added to the soil of the sprouted plants in the first batch and in the second batch, 500µl of astaxanthin was treated for four days continuously. One paper cup in each batch was maintained as the control, to which astaxanthin was not treated.

Detection of astaxanthin by Thin Layered Chromatography(TLC)

After seven days of sprouting and treatment the plants of each batch with astaxanthin were removed from the soil and were subjected to washing with running tap water. The whole plants from each batch were subjected to homogenization with acetone and a pinch of magnesium sulphate and the content was incubated at room temperature for 30 minutes in dark. The upper phase was discarded and the lower phase was used as the sample for TLC. Silica gel G was used as the stationary phase and the mobile phase used was Acetone, butanol and isopropanol in the ratio 5:2:1 with a few drops of distilled water.

Antioxidant Property Analysis of Fenugreek Sprouts

The antioxidant property of fenugreek plant was estimated by using DDPH as substrate (Wollgast *et al.*,2000). The standard vitamin C was used as control for the analysis of antioxidant activity of DPPH substrate.

RESULTS

The 13.4µg.gm^{-1} of astaxanthin was extracted by using as the acetone as solvent from the exoskeleton of *Fenneropenaeus merguiensis* (Fig.1 and 2).

Fig 1 Exoskeleton of *Fenneropenaeus merguiensis*

Fig 2 Acetone extract of *F. merguiensis* exoskeleton

The seeds of fenugreek when treated with astaxanthin, failed to sprout and the when the sprouts of fenugreek were treated with astaxanthin, the leaves of fenugreek were found to accumulate astaxanthin. The batch of leaf which were treated with 100µl of astaxanthin showed good plant growth and 500µl of astaxanthin treated plants, exhibited good accumulation of astaxanthin, but the plant growth was found to be retarded (Fig.3).

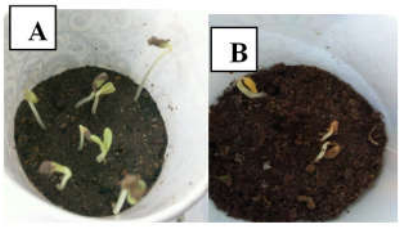

Fig 3 Astaxanthin Treated Fenugreek Plants

A. 100µl of astaxanthin treated fenugreek plant
B. 500µl of astaxanthin treated fenugreek plant

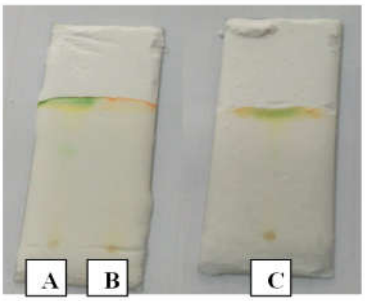

Fig 4 TLC analysis of plant pigments

A: Control fenugreek Pigments
B: Acetone extract astaxanthin from Prawn exoskeleton
C: Astaxanthin treated plants (100µl)

The fenugreek plant treated with astaxanthin and the control plant on thin layer chromatography of the plant compounds were analyzed (Fig.4). The antioxidant property of the control plant and the experimental plant were analysed with DPPH as the substrate (Table.1).

Tab 1 Estimation of Antioxidant Activity

S. No.	Culture	Asataxanthin ,µg/ml	
		2nd day	5th day
1.	Control	53%	56%
2.	Astaxanhtin treated (100µl)	65%	76%

DISCUSSION

The mainly pharmacological important compounds found in fenugreek leaves are steroids, polyphenolic compounds, amino acids and the seeds contains galactomannans, tryptophan, alkaloids, choline, vitexin, sapogenins, vitamins like A, B1, C and nicotinic acid (Mehrafarin *et al*., 2010 and Mohsen *et al*., 2012). This plant can be easily cultivated in any climatic conditions and in any soil profile and it is also known to improve the nitrogen content of the soil ((Sadeghzadeh-Ahari *et al*., 2009). According to Magda, 2017; the germinated fenugreek seeds when analysed by gas chromatography and ion-trap mass spectroscopy contains 4.93% astaxanthin and the antioxidant property was estimated as 56.30%.

In this present study, the astaxanthin treatment at a concentration of 100 µl, supported for bioaccumulation and good plant growth. The antioxidant activity of control fenugreek and treated fenugreek plants were analyzed and the antioxidant property of astaxanthin treated fenugreek showed better antioxidant activity than the control plants. Thus by the treatment of the extracted astaxanthin from the exoskeleton of *Fenneropenaeus merguiensis* plays an important role in the biofortification of the fenugreek sprouts.

CONCLUSION

The fenugreek plants that were treated with astaxanthin which was extracted from the exoskeleton of *F. merguiensis* at low concentration showed good accumulation of astaxanthin and thereby enhancing antioxidant activity of the fenugreek sprouts. This biofortified fenugreek could further enhance the biological application of fenugreek. Thus such research could help us to reduce the biological waste and also to enhance the production of crops plant with improvised biopotentials.

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