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Report on Betalain Pigments Produced by Endophyte in *Amaranthus cruentus* L.

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Abstract

The appearance of red colour pigmentation in red amaranth (*Amaranthus cruentus* L.) is due to Betalain pigment present in it. Endophytic fungus *Fusarium falciforme* is reported from this plant. Extraction studies from fungus *Fusarium falciforme* recorded the presence of Betalain pigments. Red colour of the said plant may be due to the presence of this endophyte.

Key words: *Amaranthus cruentus* L, Endophyte, *Fusarium falciforme,* Betalain

In rabbi season red amaranth (*Amaranthus cruentus* L.) is most common crop in different regions of India. This crop may increase the beauty of the field due to its reddish pink appearance. It is established that Belatain pigments are responsible for its red colour appearance [1]. *Amaranthus cruentus* L. is a flowering plant in the Amaranthaceae family that is frequently known as "red amaranth." It is an annual, pseudo-cereal with wide leaves eaten as a vegetable; it also produces high-protein grains and is an excellent feed crop [2]. It yields edible seeds, which are eaten raw, used as cereal grain, or cooked into *Rajgira Laddoo*; the leaves are also prepared in the same way as spinach [3-4]. *Amaranth* is drought resistant and grows best in warm temperatures. The presence of betalain was discovered in pigment analysis and tissue culture of *Amaranthus cruentus* L. [5]. Some betalains derivatives (16 red-violet betacyanins and 3 yellow betaxanthins) have been extracted and found in stems, leaves, and inflorescences of Amaranthaceae plants [6]. The sequence of an *Amaranthus cruentus* L. projected betalain biosynthetic gene (AcCYP76AD1 GenBank accession: KR376479.1) was acquired from NCBI databases [7].

In a separate investigation, photometric detection of *Fusarium oxyporium* betalain pigments such as Amaranthin, betanin, betalamic acid, and betaxanthin was made [8]. *Fusarium falciforme* has been identified as an endophytic fungus in *Manohot esculenta* and *Centella asiatica* [9-10]. The endophytic fungus *Fusarium falciforme* was isolated from the root of *Amaranthus cruentus* L. in this study (Ref. No NC160819). Because of their poisonous, carcinogenic, and teratogenic qualities, interest in synthetically produced pigments has waned in recent years [11]. Then there was the option of focusing on microbial origins [12]. When we compare natural pigments of plants, in nature microbes are available at large. Down streaming processing costs of microbial pigments are very les as well as their stability and shelf life also more. Such microbes are capable of a wide range of functions, including anti-inflammatory, immune-regulatory, anticancer, and antioxidant ones. For instance, Manascus, Arpink, and β-carotene were obtained from *Penicillium oxalicum*, *Blakeslea trispora*, and different microorganisms, respectively; all of them are utilized in the food sectors [13]. In more recent times, the degradation of synthetic pigments has been greatly accelerated by physicochemical stress factors brought on by food production, preservation, storage, and preparation, interactions with other food ingredients, and absorption at the various interfaces (such as between aqueous and lipid or solid phases) present in the food [14].

MATERIALS AND METHODS

From the fields in the Limbala region, MIDC Road, Hingoli, district in Maharashtra, India. healthy *Amaranthus cruentus* L. plants were picked (MS). The plant's roots were cleaned individually for 10 to 15 minutes under running water. Four measures were used to sterilize the surface. Samples were first washed with tap water. After being submerged in 70% alcohol for 1 minute, 3.5% sodium hypochlorite for 2 minutes, 70% ethanol for 30 seconds, and then sterile water for 1 minute, the samples were then rinsed until no traces of the prior solution remained.

Using Potato Dextrose Agar (PDA), endophytic fungi from *Amaranthus cruentus* L. were isolated. The samples that had been surface sterilized were divided into little pieces and put on Petri plates with Potato Dextrose Agar (PDA) medium. They were then cultured for a week at 27 °C. Following a week of incubation, fungal isolates were screened by subculturing them on Potato Dextrose Agar (PDA) plates. Young mycelia were allowed to develop in 250ml of potato dextrose broth before being cultured for 14 days at a temperature of 26-28 °C [15].

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After the incubation time, ethyl acetate was added as an organic solvent to homogenize the fungus' fermented broth. Equal amounts of the solvents were added to the filtrate, which was then thoroughly mixed for 10 minutes and left for 5 minutes to create two transparent, immiscible layers. Using a separating funnel, the solvent's top layer that contained the extracted chemicals was separated. The crude metabolite was produced by evaporating the solvent and drying the resulting chemical in a rotator vacuum evaporator [16]. The process is depicted in (Fig 1a-g).

Fig 1 (a-g) a: Red amaranth Plant, b: Leaves, c: Roots, d: isolated endophyte on PDA, e: seperation of pigment, f: extracted supernatant, g: drying pigment

RESULTS AND DISCUSSION

Amazing colours from several chemical classes, including carotenoids, melanins, flavins, phenazines, quinones, and monascins, are produced by filamentous fungus [17]. Carotenoids and polyketides are considered to be fungus pigments [18]. The majority of fungi create water-soluble pigments, which are perfect for industrial production since they can readily be scaled up in commercial fermenters and extracted without the need of organic solvents [19]. The demand for eco-friendly natural dyes derived from sustainable resources is being promoted due to the dangers that synthetic colourants provide to both the environment and human health.

The potential of fungal pigment, one of the microbial pigments, is now being investigated for textile dyeing as well as a number of other uses. A remarkable variety of colours from various chemical groups are produced by fungus. When used on textile materials, fungus pigments have shown to be safe and to have sufficient colour stability to tolerate changes in temperature, pH, and additives that affect colour fastness. The usage of fungal pigments in the textile industry has increased as a result of technological advancements, genetic engineering techniques for strain enhancement, and vast fungal variety. However, in order for them to be used commercially, fungal pigments need to undergo quality and toxicity tests in order to receive regulatory permission before being on sale [19].

Fungal mycelium of *Fusarium falciforme* was allowed to grow in PDB, the result obtained recorded presence of pigment in PDB. Using separating funnel, the pigments which were allowed to get dissolved in organic solvent ethyl acetate were separated (Image). Further Cultivation, extraction and purification of fungal pigment is needed. The authors have strong belief that the pigments produced by red amaranth may be due to presence of fungal endophyte in it. Elaborative research will help understand the role of *Fusarium falciforme* in red amaranth. If it has to be due to secondary metabolites produced, it will definitely put more opportunities in potential to use of it to produce pigment at large scale.

CONCLUSION

The chosen fungus, *Fusarium falciforme*, has the ability to manufacture dye-able pigment. To get the greatest pigmentation, the growing conditions for this fungus may be improved. These dyes can be used on fabrics, but it's important to look for any negative effects they may have on the tensile strength of the material. More pigments were discovered; however, they should not be harmful to human skin. The study's findings suggest that fungus may be a source for developing colours that are suitable for use in both food and textiles. Because it is possible to standardize the fermentation conditions for maximizing pigmentation, these natural dyes may be manufactured commercially on a large scale for less money and with less harm to the environment.

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