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SHORT COMMUNICATION

ESTIMATION OF BIXIN IN SEEDS OF *BIXA ORELLANA* L. FROM DIFFERENT LOCATIONS IN WESTERN MAHARASHTRA

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Seeds of *Bixa orellana* L. were collected from four different locations of Maharashtra (Dapoli, Kolad, Powai and Savle). The pigments were extracted from the seeds by direct extraction with oil, direct extraction with aqueous alkali and indirect extraction with organic solvents (chloroform, chloroform: ethanol, ethyl acetate) and were assayed using spectrophotometric method. Castor oil yielded comparatively smaller amount of bixin, which was maximum in Kolad samples. Aqueous alkali (pH13) extracted 0.4-1.25%, nor-bixin, maximum was in the sample collected at Savle. Chloroform could extract maximum bixin. Mixture of chloroform and ethanol was not very efficient in extracting bixin. Seeds from Dapoli had maximum bixin (chloroform extraction) and Kolad had maximum of minor carotenoids (ethyl acetate extraction).

***Key words*: Bixin, *Bixa orellana*, nor-bixin.**

Increased awareness of the use of natural colorants for food, cosmetics, leather and textiles industry has put a great demand on use of annatto colorant. Among the naturally occurring colorants, annatto ranks second in economic importance (Ghiraldini, 1989). Annatto extract is taken from the seeds of a tropical tree known as *Bixa orellana*. The principal coloring component of annatto is a carotenoid known as bixin. Bixin is a highly desired coloring agent for food specially dairy products.

Phytogeographic locations, their climatic and edaphic conditions are known to exert some effect on secondary metabolite production in plants. *Bixa orellana* is a tropical plant that grows equally well in lowland and mountainous region or areas of higher elevations. This plant is a source of carotenoid type food colorant, which are extracted and available in market as oil soluble formulations or aqueous extracts or solvent extracted dried powder. In Maharashtra this tree is found growing in the wild in western region on both hills and plains. This work is an attempt to find out the effect of different phyto-geographic conditions on pigment content.

Mature seeds from red capsule variety of *Bixa orellana* L. were collected in winter from 4 different locations (Dapoli, Kolad, Powai and Savle) of Western Maharashtra. Dapoli, Savle and Powai are hilly zones whereas Kolad is in plains. All the 4 places are situated at 18 to 20 N latitude and 72.5 to 73.5 S longitude. Powai and Savle are comparatively more humid zone than Kolad and Dapoli.

For extraction of bixin morphologically similar seeds (i.e. seeds having similar color, no wrinkles and 2-4 mm diameter were selected and dried over night in an oven at 40^{o}C.

Three different processes were used to extract the pigment from 10 gm dried seeds collected from each location i.e. (i) direct extraction into castor oil, (ii) direct

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extraction into aqueous alkali at 3 different pH 8, 10 & 13 and (iii) indirect extraction with organic solvents as per Preston and Richard (1980) method.

(i) For castor oil extraction 10 gm seeds were crushed and incubated in 50 ml castor oil at 150ºC for 6 hrs (Srinivasulu and Mahapatra 1982). Oil was cooled to room temperature and then decanted.

For analysis of bixin content, 20 mg oil extract was dissolved in chloroform.

The chloroform layer was separated in a separating funnel and bixin content was analysed using Srinivasulu's (1996) spectrophotometric method:

$$\text{Bixin Content (\%)} = \frac{\text{Test absorbance x 1 x Dilution of Sample x 100}}{\text{3200 x Dilution of Standard x Weight of Sample}}$$

Where E 1cm at 1% = 467 nm = 3200

(ii) For direct extraction in aqueous alkali, 10 gm seeds were extracted with 40 ml alkaline water set at pH 8, 10 and 13; at 37ºC for 48h following Smith and Porter (1983) method. The nor-bixin extracted with alkaline water, was precipitated by decreasing the pH to 3 using sulfuric acid. The extract was dried in a vacuum oven at 30ºC and 30 mm pressure for 6h. Extraction was carried out 7 times to extract maximum nor-bixin.

For analysis, 20 mg extract was dissolved in 50 ml aqueous KOH solution at 70ºC for 15 min. The solution was centrifuged at 2000 rpm for 15 min and nor-bixin was estimated at 480 nm (Srinivasulu 1996).

$$\text{Bixin Content (\%)} = \frac{\text{Test absorbance x 1 x Dilution of Sample x 100}}{\text{2870 x Dilution of Standard x Weight of Sample}}$$

Where E 1 cm at 1% = 480 nm = 2870.

(iii) For indirect extraction in organic solvent, three different solvents were used

(a) 40 ml of 1:3 chloroform and ethanol (Bhalkar and Dubash, 1983) was added to 10 gm seeds and kept at 37ºC for 48 h. The extract was dried in vacuum fume hood for 6 h and then in an oven at 105ºC for 48 h. Bixin content was analysed using chloroform as solvent and Srinivasulu's (1996) method at 467 nm.

(b) 10 gm seeds were first defatted by keeping in 60 ml n-hexane at 60ºC for 6 h. 60 ml ethyl acetate was added to defatted seeds and extraction was done at 80ºC for 6 h. Extract was dried and bixin was analysed by spectrophotometric method at 467 nm (Srinivasulu, 1996).

(c) To 40 ml chloroform (Avila *et al.*, 1982), 10 gm seeds were added and kept at 37ºC for 48h. The extract was dried in a vacuum fume hood for 6h and then in an oven at 105ºC for 48h. Bixin content was analysed using chloroform as solvent at 467 nm (Srinivasulu, 1996).

Oil extract: Bixin occurs in oil soluble annatto in *cis* and *trans* forms, known as α and β bixin, respectively (Lancaster and Lawrence 1995). Least amount of bixin was extracted with castor oil though it is the most desired form of extraction because liposoluble bixin is used for coloration of many dairy products (Table 1).

Oil extracts from seeds of Kolad and Dapoli had maximum amount of bixin, 0.81% and 0.75%, respectively (Table 1, Fig. 1) and are darker in color than the oil extracts from the seed of Savle and Powai area which has only 0.18 and 0.12% bixin, respectively. It is also interesting to note that though seeds collected from Powai area were very dark (almost blackish maroon), the oil extract was very light having lowest amount of oil soluble bixin. Bixin is not present in the seed coat, but underneath it between seed coat and cotyledon.

The usable concentration of bixin in oil extract is in the range of 0.1 to 1.0%: hence oil extracts from all the samples can be sold directly as an oil formulation.

Table 1. Bixin content in seed samples collected from 4 different locations and extracted in different mediums

Locations	% bixin extracted from seeds in			
	Castor Oil	chloroform: ethanol	ethyl acetate	chloroform
Dapoli	0.75	4.69	15.82	36.5
Kolad	0.81	1.2	20.3	28.5
Powai	0.12	0.06	4.5	31.08
Savle	0.18	0.04	4.72	20.67

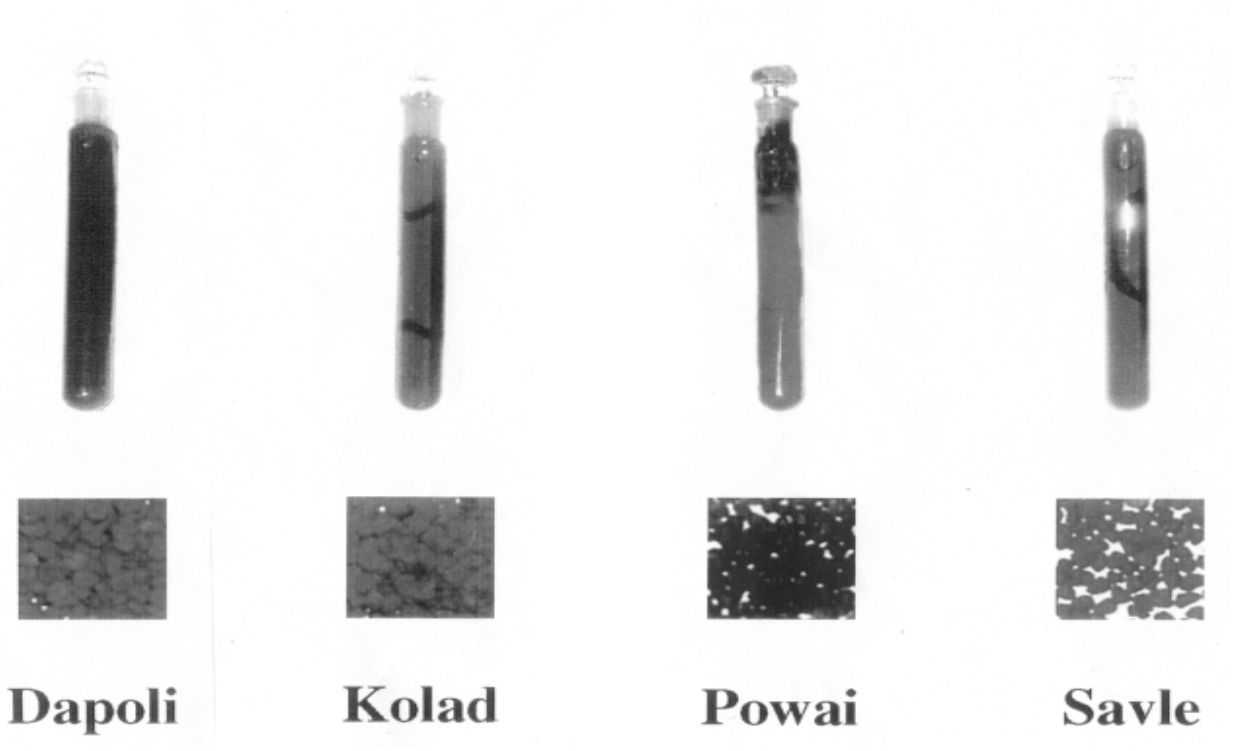

Fig. 1. *Bixa orellana* L. seeds collected from different locations of Maharashtra and bixin extracted from them in castor oil

Alkaline aqueous extract: Water soluble annatto contains α and β nor-bixin (Scotter, 1995). Amongst the three different alkaline pH, the most suitable for not-bixin extraction was pH 13 (Table 2). The nor-bixin content was maximum in the sample collected from the hilly areas of Dapoli, Powai and Savle. The samples collected from the plains of Kolad had comparatively less nor-bixin.

Organic solvent extract: None of the solvents used in annatto extraction are of edible type, hence are subsequently removed. As compared to previously

Table 2. Nor-bixin content in seed samples collected from 4 different locations extracted with aqueous alkali.

Locations	% nor-bixin extracted from seeds in aqueous alkali having		
	pH 8	pH 10	pH 13
Dapoli	0.45	0.8	1.17
Kolad	0.25	0.35	0.42
Powai	0.24	0.48	0.83
Savle	0.35	0.75	1.25

mentioned two solvents (oil and alkaline water) organic solvents produced highly concentrated extracts consisting mainly of 9'-*cis*-bixin along with much lesser quantities of 9'*cis*-nor-bixin (Scotter *et al*., 1998).

Chloroform was the best solvent as it could extract maximum amount of bixin; followed by ethyl acetate and minimum extraction was in a mixture of ethanol and chloroform (Table 1). None of the organic solvent showed any distinct effect of altitude on bixin content in the seeds. However, ethyl acetate extracts showed the same trend of results as that of the castor oil. Although chloroform could yield maximums bixin, as all the isomers of bixin are soluble in chloroform, it contains toxic residue in the extracts hence bixin extracted in ethyl acetate from defatted seeds is used as food colorant.

Bixin extracted with organic solvents in normally used as a dye for textile (Webb *et al*., 1961) and also in paint, varnish and lacquer industry.

Various workers have done analysis of bixin and nor-bixin content for a very long time in different parts of the world. They (Preston and Rickard, 1980; Avila *et al.*, 1982; Srinivasulu and Mahapatra, 1982; Bhalkar and Dubash, 1982 and Scotter *et al*., 1998) have reported varying amount of bixin content from seeds. Whether it was due to extraction method or seeds collected from different locales that produced varying data has been our line of interest. Moreover since research was supported and funded by an industry interested in bixin production, it was desired to see from which locale better bixin producing seeds could be obtained.

In conclusion it can be said that there is a distinct impact of phytogeographic conditions specially altitude of the area on nor-bixin content. So far as bixin content is concerned, it varied in seeds collected from different locations, moreover, different organic solvents also showed variation in extracted bixin content.

All the data suggest that naturally growing *Bixa orellana* trees in the Western region of Maharashtra can be taken and cultivated for commercial exploitation of bixin production.

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