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*Full Length Research Paper*

# Mycoflora associated with seeds of *Bixa orellana* L.

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**Commercially important natural food grade annatto dye is obtained from seeds of tropical plant *Bixa orellana*. Fungal contaminations of seeds adversely affect the quality of seeds. We have investigated seed mycoflora of annatto seeds obtained from standing crop of *B. orellana*. Significant fungal contamination was detected in analyzed seed samples. Fungi most frequently isolated and identified were *Aspergillus* sp., *Alternaria* sp., *Penicillium* sp., *Cladosporium* sp., *Fusarium* sp., *Rhizopus* sp., *Stachybotrys atra,* and *Syncephalastrum racemosum.* This is the first report of mycoflora of annatto seeds. This study will provide knowledge of fungal incidence on annatto seeds and in future to find suitable remedy to avert the storage loss by fungi.**

**Key words:** Annatto, *Bixa orellana* L., mycoflora, incidence, mycotoxins.

## INTRODUCTION

Annatto seeds obtained from *Bixa orellana* L. (Bixaceae) are actual source of reddish orange dye that is present on its portion and is one of the important food grade colourant (Mercadante et al., 1999) used particularly for coloring dairy products apart from its wide usage in baking, confectionary products, cosmetics, dying leather etc (Chattopadhyay et al., 2008). In view of its economical importance at global market (Levy and Rivandeneira, 2000) the seed quality from percent bixin content is very important (Iqbal, 1993). Apart from this, the healthy nature of seeds is also vital otherwise fungal incidence adversely affects its price. Since last five decades a wealth of literature is available on incidence of fungi on various agricultural commodities (Hareesh Vardhan Rao et al., 1995; Giridhar and Reddy, 1997, 1999, 2006; Zaboli et al., 2011) including spices and dry fruits. Such reports are either field based or under different storage conditions. Moreover, these moldy seeds of annatto that are discarded during harvesting from field spread to the healthy plants in subsequent season which leads to severe loss to the growers and also seed-borne diseases have been found to affect the growth and productivity of crop plants (Dawson and Bateman, 2001). Apart from this, in traditional medicinal practices of African countries, various ethno-botanical applications were attributed to annatto seeds (Parrotta, 2001). Consumption of such mould infected food stuff has been a matter of serious concern all over the world (Sweeney and Dobson, 1998) and the same may be applicable to annatto dye too as the dye is extracted by using organic solvents like that of mycotoxins. Extensive research on *B. orellana* plant carried out by various researchers mainly confine to annatto pigment, downstream processing and biotechnological improvement of this plant (Satyanarayana et al., 2003; Rodríguez-Ávila et al., 2010). Studies pertaining to fungal incidence on seeds during harvesting time are not available and the same is warranted in view of annatto dye usage for food and cosmetic applications. In the present communication we are reporting the incidence of fungi on seeds of *B. orellana* standing crop.

## MATERIALS AND METHODS

### Seed sample collection

Annatto seeds were collected from about to dehiscent capsules (70 days after flowering) from standing crop of *B. orellana* L. (Bixaceae). These seeds were used for the isolation and detection

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**Table 1.** Incidence of fungi on seeds of *Bixa orellana* L.

| S/N | Name of the fungus | Incidence (%) | Frequency (%) |
|---|---|---|---|
| 1 | *Alternaria alternata* | 8.54 | 48 |
| 2 | *Aspergillus flavus* | 6.83 | 60 |
| 3 | *Aspergillus fumigatus* | 4.70 | 32 |
| 4 | *Aspergillus niger* | 2.56 | 52 |
| 5 | *Aspergillus ochraceus* | 1.70 | 16 |
| 6 | *Aspergillus terreus* | 5.55 | 20 |
| 7 | *Cladosporium cladosporioides* | 5.55 | 36 |
| 8 | *Cladosporium oxysporum* | 5.55 | 32 |
| 9 | *Curvularia lunata* | 1.70 | 12 |
| 10 | *Curvularia* sp. | 2.99 | 4 |
| 11 | *Fusarium equeseti* | 5.55 | 12 |
| 12 | *Fusarium moniliforme* | 5.12 | 24 |
| 13 | *Fusarium oxysporum* | 6.41 | 16 |
| 14 | *Penicillium chrysogenum* | 5.55 | 28 |
| 15 | *Penicillium citrimum* | 3.84 | 20 |
| 16 | *Penicillium notatum* | 0.85 | 4 |
| 17 | *Periconia* sp. | 5.98 | 12 |
| 18 | *Rhizopus oligosporus* | 2.13 | 48 |
| 19 | *Stachybotrys atra* | 0.85 | 4 |
| 20 | *Sterile mycelia* | 2.56 | 20 |
| 21 | *Syncephalastrum racemosum* | 3.84 | 24 |
| 22. | Unidentified colonies | 5.98 | 8 |

of seed-borne fungi. Seed samples (25 samples) were randomly picked from 100 matured open capsules that collected from *B. orellana* standing crop (from 25 plants) and used for the experiment. These seeds were collected in sterilized polythene bags and condition of the samples and details of storage were recorded.

#### Culture medium and experimental conditions

Blotter method recommended by international seed testing association (Anonymous, 1985) and dilution plate method (Waksman, 1922) were used for the isolation of fungi. In blotter method, seeds were kept on two layers of moistened blotter papers placed in 90 mm diameter Petri plates (25 No.) with 25 seeds/plate. The plates were incubated for 7 days under dark. In dilution plate method, 10 g of seeds were taken into 250 ml conical flask containing 100 ml of sterile distilled water and kept on shaker for 20 min. Then the same was subjected to serial dilution and appropriate dilutions was subsequently placed in known quantity to the sterile petriplate, followed by pouring of sterilized Asthana & Hawker's medium (Glucose-5 g; $KNO_3$-3.5 g; $KH_2PO_4$-1.75 g; $MgSO_4.7H_2O$-0.75 g and distilled water 1000 ml) with immediate stirring for uniform spreading of inoculum. The plates were incubated for 10 days at room temperature (28 ± 1°C) under dark conditions. The fungi were isolated and identified with the help of available literature (Barnett and Hunter, 1972; Domsch et al., 1980, Frisvad and Filtenborg, 1983). The percentage of incidence and frequency were calculated by employing the following formulae.

$$\% \text{ of incidence} = \frac{\text{No of colonies of species in all plates}}{\text{Total no of colonies of the all the species in all plates}} \times 100$$

$$\% \text{ of frequency} = \frac{\text{No of observations in which fungal species appeared}}{\text{Total no of observations}} \times 100$$

## RESULTS

### Fungi incidence on annatto seeds

Table 1 reveals that there was fairly high incidence of mould infestation on annatto seed samples based on dilution plate method. A total of 20 species belonging to 10 genera were associated with seeds (Figure 1). This may be attributed to the warm, humid environmental conditions of the locality. The most prominent fungi in different samples comprised species of *Aspergillus, Fusarium* and *Penicillium* followed by *Rhizopus oligosporus* etc. The percentage incidence of *Alternaria altenata* (8.54) was at maximum followed by *Aspergillus flavus* (6.83), *Fusarium oxysporum* (6.41), *Aspergillus terreus* (5.55), *Pencillium chrysogenum* (5.55), *Periconia* sp., (5.25) and *Cladosporium cladosporioides* (5.55).

In blotter technique too, significant number of fungi were recorded a total of 15 species belonging to 6 genera were found (data not given), viz., *A. alternata*, *Aspergillus* sp., *Cladosporuim* sp., *Fusarium* sp., *Penicillium* sp. and *R. oligosporus*. The percent frequency of *A. flavus* and *Aspergillus niger* were high followed by *R. oligosporus* and *A. alternata*.


**Figure 1.** Fungal infestation of fruit capsule and seeds of *Bixa orellana* L. (A): Healthy dehisced fruit bunch; (B): dried fruit capsule with fungal infestation on its surface (C): dehisced fruit capsule with infected seeds.

## DISCUSSION AND CONCLUSION

It is of great concern that many of the isolated and identified species are known to produce mycotoxins (mycotoxigenic nature not investigated) particularly aflatoxins (*A. flavus*), terreic acid (*A. terreus*), trichothecenes (*Fusarium* sp.), and citrnin, cyclopiazonic acid (CPA) etc produced by *Pencillium* sp. as reported earlier (Betina, 1989; Giridhar and Reddy, 2001; Hell and Mutegi, 2011). Annatto seeds especially collected from warm and humid climates may be of high risk because of these fungi and an improper processing condition may enable their survival, followed by mycotoxin contamination in annatto extracts. In our study, the incidence of *A. alternata* on annatto seeds was also significant which is a common phenomena as observed in oil seeds, cereals and pulses (Rathod and Chavan, 2010). The unseasonal rains especially during the fruit maturation and dehiscence stage (September to November) exacerbate the mould incidence on annatto seeds.

Furthermore, mixing of annatto dye to various food products can serve as a source of mycotoxins, which are in general low concentration and challenging to detect. Apart from this, seed waste (after dye extraction) is used in some places as feed in view of its high protein content (Senthil Kumar et al., 2007) in such cases the mould infected seeds are of concern to the health of animals. Upon storing of these mold infested seeds, there is also a possibility of continuous growth in some of the storage fungi and increase in the moisture content (Chakrabarty, 1987), this leads to predominance of certain storage fungi such as *A. niger* and *A. flavus* and *Mucor* sp. and further losses to the annatto seed quality as in case of Jatropha seeds (Jayaraman et al., 2011). Apart from this, there is further spread of these fungi to the subsequent harvests which may lead to yield loss, bio-deterioration and chemical value of annatto seeds as in case of pulses, cereals and oil seeds (Rathod and Chavan, 2010). Moreover, seed borne fungi may cause seed rot, reduction of germination capacity and also results in development of disease at later stages of plant growth hence, seed-borne mycoflora of annatto seeds need serious attention.

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