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SHORT COMMUNICATION

Fungal contamination of stony endocarp (Rudraksha) of *Elaeocarpus* spp. from three different countries

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The study deals with fungal contamination of stony endocarp (Rudraksha) of *Elaeocarpus* spp. collected from India, Nepal and Indonesia. For mycological analysis, the agar test method including surface sterilized and non surface sterilized samples were used. Eight species belonging to four different genera of fungi were isolated. The genera isolated were *Aspergillus* (four species), *Penicillium* (two species), *Alternaria* (one species) and *Rhizopus* (one species). The study emphasizes that fungal contamination would deteriorate the quality of stony endocarp (Rudraksha) of *Elaeocarpus* spp. and also affect its marketing. This is the first record of mycoflora study from stony endocarp (Rudraksha) of *Elaeocarpus sphaericus* (Gaertn.) K. Schum. from India and Nepal and *E. ganitrus* Roxb. from Indonesia.

Keywords: Rudraksha, *Elaeocarpus sphaericus*, *Elaeocarpus ganitrus*, Endocarp, Fungal contamination, Deterioration, Marketing.

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Introduction

Rudraksha (from Sanskrit "Beads of Rudra") is a common name of 5-lobbed stony endocarp derived by plants from genus *Elaeocarpus* section Ganitrus (Gaertn.), being the largest genus in the Elaeocarpaceae comprising 350 species distributed from Madagascar in the west to Fiji and Hawaii in the east[1].

Rudraksha, the hard endocarps of *Elaeocarpus* L. drupes plays a significant religious importance and used by Hindu and Buddhism believers, as well as by Tibetan and Nepalese Shamans, for manufacturing of prayer beads, temple decoration, ritual objects and in traditional and village medicine based on "Ayurveda"[2]. The fruit stone (seed kernel) is sweet, cooling, emollient, cerebral, sedative, expectorant, liver tonic and febrifuge. It is useful in treating epileptic fits, melancholia, manic conditions, mental disorders, convulsions, insomnia, cephalalgia, hepatopathy, hypertension, bronchitis, fever and vitiated conditions of *kapha* and *vāta*[3]. The fruit of *E. sphaericus* (Gaertn.) K. Schum. is used by locals in Nepal for treatment diseases of the head, epileptic fits and mental disorder and the beaded endocarps are used as garlands and assumed to reduce blood pressure[4]. The main market of Rudraksha in the world is India (more than 85%), but interest to Rudraksha is growing worldwide at China, Japan, Russia, Europe and USA. Only in India, market turnover is around 12,000,000 $ per year. Today the sale of Rudraksha is varied and wide from: simple street vendors to jewelers, the television and on the Internet, has all conspired to create a massive market.

The stony endocarp of *Elaeocarpus* drupes are found after removing the fruit pulp. The endocarp is rough in texture, with its surface divided into segments, by ridges running from top to bottom. They are commonly 5-celled, strongly tubercled and marked with as many longitudinal furrows, as there are cells in the stone. Each locule normally houses a seed. Thus, a 5-celled ovary may produce between from 1 to 5 seeds (and often none), depending upon the abortion of ovules during its development into seeds[5]. In the scripture Srimad-Devibhagavatam[6], it is described that the best Rudraksha must be: round (circular), have uniform shape, be smooth, yet hard to the touch, with thorns on the surface, they must be unbroken and fissure-less, fully developed. However, during collection of Rudraksha, the authors have experienced that most of the beads that are damaged by insects or worms, broken, without thorns, swells, non-circular and very dirty due to fungal contamination are not good to use. Fungal contamination on stony endocarp of Rudraksha also causes serious threat to the marketing of Rudraksha as it loses attractiveness to the buyers due to change of color (from attractive golden-copper to grayish or blackish) and texture. After a year of storage fruits are not suitable for sale and causes big economical loss to the farmers and sellers.

Hence, the present study was conducted to record the mycoflora on stony endocarp of Rudraksha

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collected from three different countries having a large cultivation and market of Rudraksha. The stony endocarps of *E. sphaericus* (Gaertn.) K. Schum.were collected from India and Nepal and the same of *E. ganitrus* Roxb. were collected from Indonesia. There are some reports on fungal contamination of *E. floribundus* Blume from Assam, India[7] and Papua new Guinea[8]. However, no work has been done so far to explore the fungal contamination of stony endocarps which are of much economic value. Therefore, this study was focused on isolation and identification of fungi from stony endocarp responsible for its deterioration and loss.

Methodology

Collection of samples

In Nepal, the stony endocarps of *E. sphaericus* were collected from various farmers of districts Sankhuwasakha, Bhojpur and Dhankuta in the month of October, 2011. In India, the stony endocarps of *E. sphaericus* were collected from the farmers of Bagheykhola (Sikkim) and Darjeeling (West Bengal) during October-November, 2011. The samples were sun dried and packed inside jute bags. In Indonesia, the endocarps of *E. ganitrus* were purchased from a local seller of the market of Jakarta of Java in the month of December, 2011. All the samples were collected in sterilized polythene bags to avoid further microbial contamination.

Mycological analysis

The mycoflora of stony endocarp was examined using the agar plate method[9] as recommended by International Seed Testing Association[10]. Two methods were followed: Surface sterilization method and without surface sterilization method. In both methods the medium used was Potato dextrose agar (PDA) (potato, 200 g; dextrose, 20 g; agar, 15 g; distilled water, 1000 mL; pH, 5.6 ± 0.2). In Surface sterilization method, 50 randomly selected stony endocarps from each country were cut into two pieces by sterilized cutter and were surface sterilized (1% solution of sodium hypochlorite) and rinsed in three changes of sterile distilled water. Each cut endocarps were then placed at the centre of Petri plates containing PDA medium and incubated for 7 days (28±2°C). For without surface sterilization method, the same procedure was applied except that the half cut endocarps were directly placed at the centre of Petri plates containing PDA medium.

Isolation and identification of fungal species

Different fungal colonies associated with endocarps were counted by examining plates. Visually and morphologically different mould colonies were sub-cultured on PDA medium. The isolated fungal species were identified on the basis of cultural and morphological characteristics following Raper and Fennel[11], Pitt[12] and Domsch *et al*[13]. The cultures of fungal isolates were maintained on PDA slants.

Results and Discussion

A total of eight fungal species belonging to four genera were isolated from all the samples (Table 1). The genera with the highest number of species isolated was *Aspergillus* (four species), followed by *Penicillium* (two species), *Alternaria* (one species) and *Rhizopus* (one species). The species isolated were *A. niger* van Tieghem, *A. flavus* Link, *A. luchuensis* Inui and *A. terreus* Thom. (from the genus *Aspergillus*); *P. citrinum* Thom. and *P. italicum* Wehmer (from the genus *Penicillium*), *A. alternata* (Fr.) Keissl. (from the genus *Alternaria*) and *R. nigricans* Ehrenberg (from the genus *Rhizopus*). *Rhizopus nigricans* was isolated from all six types of the samples showing the predominancy over other species. *Aspergillus flavus* was isolated from all types except the surface sterilization method from the samples of Nepal. *Aspergillus niger*, *A. luchuensis* and *Penicillium citrinum* were isolated from four types, *Aspergillus terreus* from three types and *Penicillium italicum* and *Alternaria alternata* from two types of samples (Table 1).

Table 1—Fungal association with the Rudraksha samples from India, Indonesia and Nepal

Fungi	India		Indonesia		Nepal	
	SS	WSS	SS	WSS	SS	WSS
Aspergillus niger	+	+	+	+	-	-
A. flavus	+	+	+	+	-	+
A. luchuensis	+	+	+	+	-	-
A. terreus	+	+	+	-	-	-
Penicillium citrinum	+	+	-	-	+	+
P. italicum	-	-	+	+	-	-
Alternaria alternata	-	-	+	+	-	-
Rhizopus nigricans	+	+	+	+	+	+

*SS: Surface sterilized;

*WSS: Without surface sterilized

Table 2—Number of individual fungal species isolated from Rudraksha

Country	Method	Fungi isolated	Total nos.
India	SS	A. n. (68), A. l. (58), A. f. (56), P.c. (16), A. t. (6), R. n. (5)	209
	WSS	A. n. (72), A. l. (64), A. f. (64), P.c. (16), A. t. (8), R. n. (8)	232
Indonesia	SS	A. f. (72), P. i. (72), A. a. (16), A. l. (7), A. n. (6), R. n. (6), A. t. (4)	181
	WSS	A. f. (136), P. i. (96), A. a. (48), A. l. (8), A. n. (8), R. n. (7)	303
Nepal	SS	P. c. (63), R. n. (48)	111
	WSS	P. c. (72), R. n. (48), A. f. (16)	136

Total: 1172

Values in (*) are the number of fungi isolated.

SS: Surface sterilized; WSS: Without surface sterilized

A. n. : *Aspergillus niger*; A. f. : *Aspergillus flavus*; A. l. : *Aspergillus luchuensis*; A. t. : *Aspergillus terreus*; P. c. : *Penicillium citrinum*; P. i. : *Penicillium italicum*; A. a. : *Alternaria alternata*; R. n. : *Rhizopus nigricans*

Table 3—Percent occurrence of fungus associated with the Rudraksha samples from India, Indonesia and Nepal

Fungi	India		Indonesia		Nepal	
	SS	WSS	SS	WSS	SS	WSS
Aspergillus niger	32.54	31.03	03.31	02.64	00.00	00.00
A. flavus	26.79	27.59	39.78	44.88	00.00	11.76
A. luchuensis	27.75	27.59	03.87	02.64	00.00	00.00
A. terreus	02.87	03.45	02.21	00.00	00.00	00.00
Penicillium citrinum	07.66	06.90	00.00	00.00	56.76	52.94
P. italicum	00.00	00.00	39.78	31.68	00.00	00.00
Alternaria alternata	00.00	00.00	08.84	15.84	00.00	00.00
Rhizopus nigricans	02.39	03.45	03.31	02.31	43.24	35.29

SS: Surface sterilized;
WSS: Without surface sterilized

A total of 1172 fungal colonies were isolated from all the samples (Table 2). In surface sterilization method, 209, 181 and 111 fungal colonies were isolated from the samples of India, Indonesia and Nepal, respectively. In without surface sterilization method, 232, 303 and 136 fungal colonies were isolated from the samples of India, Indonesia and Nepal, respectively. In Indian samples ***Aspergillus niger*** was found to be dominant fungi followed by ***A. luchuensis***, ***A. flavus***, ***Penicillium citrinum***, ***Aspergillus terreus*** and ***Rhizopus nigricans***. In Indonesian samples ***Aspergillus flavus*** was isolated as the dominant fungi followed by ***Penicillium italicum***, ***Alternaria alternata***, ***Aspergillus luchuensis***, ***A. niger***, ***Rhizopus nigricans*** and ***Aspergilus terreus***. In Nepalese samples ***Penicillium citrinum*** was identified as the dominant fungi followed by ***Rhizopus nigricans*** and ***Aspergillus flavus*** (Table 3).

The results indicate that all the selected samples were heavily contaminated with the different mould species. Samples from each country showed more or less similar mycoflora in both surface sterilization method and without surface sterilization method, however, the number of isolates were less in surface sterilization method in comparison to without surface sterilization method. The findings of the present investigation emphasize variation in extent of fungal association with Rudraksha collected from different countries which might be because of their climatic conditions, storage practices, moisture content and chemical nature of the substrate[14]. As most of the isolated fungal species were saprophytic in nature, their invasion to the Rudraksha would have occurred after harvesting.

Conclusion

The fungal association will deteriorate quality of Rudraksha and will also affect its chemical profile, thereby affecting their medicinal efficacy. Hence, proper care should be taken during post harvest storage of the Rudraksha to avoid fungal contamination.

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