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Full Length Research Paper

Role of seed mycoflora on seed germination of *Oroxylum indicum* (L.) Vent. in Kumaun region of Indian Central Himalaya

B. J. Pande* and R. C. Gupta

Department of Botany, Kumaun University, S. S. J. Campus, Almora (Uttarakhand), India.

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A number of fungi was isolated from the seeds of *Oroxylum indicum* (L.) Vent. The mycoflora associated with the seeds of this tree has not been previously reported from Kumaun region of Indian Central Himalaya. During the course of the study, mycoflora such as *Fusarium solani*, *Aspergillus niger*, *Aspergillus nidulens, Penicillium* sp., *Trichoderma harzianum*, *Alternaria solani*, *Alternaria alternata, Curvolaria lunata*, *Stachybotryis chartarum*, *Acremonium* sp., *Rhizoctonia solani*, *Chaetomium globosum*, *Cladosporium cladoporoides* and *Torula allii* were isolated from the seeds.

Key words: *Oroxylum indicum* (L.) Vent; Seed Mycoflora; Kumaun; Indian Central Himalaya.

INTRODUCTION

Oroxylum indicum (L.) Vent. commonly known as Shyonak in Sanskrit, Indian Trumpet Flower and Midnight Horror in English, Aralu in Hindi and Pharkat in Kumaun, is a deciduous tree belonging to the family Bignoniaceae. It is an indigenous tree in tropical Asia, found growing in India, Ceylon, Malaya, Cochin, China, Philippines and Indonesia (Anonymous, 1972 vide Zaveri and Jain, 2010). It is a small glabrous tree having leaves opposite, large, bi or tri- pinnate. Flowers large in terminal long pedunculate robust racemes, purplish or white. Fruits large, flat, sword shaped capsules full of many flat and papery thin seeds with broad membranous wings.

There is a growing interest in seed pathology in developing countries both in terms of testing seeds for quality and in terms of seed research in the field of seed-borne fungi (Neergaard, 1975). The fungi may attack the seeds which result in the form of seed abortion, shrunken seeds, reduced seed size, seed rot, sclerotisation, seed necrosis, seed discolouration, reduction or complete loss of germ inability and physiological changes (Shetty and Prakash, 1994). Seeds are carriers of several plant pathogens which are responsible for severe diseases leading to considerable loss of crop yield (Neergaard, 1976 vide Janardhanan, 1994). Frequent failures of germination are mainly due to fungal infection of seeds.

The fungi invade the outer seed coat, endosperm and embryo. Seeds which carry seed-borne infection produce diseased seedlings resulting in massive destruction due to seedling blights (Janardhanan, 1994). A large number of seed-borne fungi produce toxic metabolites which often kill the embryo (Vidyasekaran et al., 1970 vide Janardhanan, 1994). The media (germination substrate) also play a significant role in germination because seeds have characteristic requirements as to the amount of moisture and oxygen needed for germination (Swati, 2006).

During the course of field study I was surprised to see sparse distribution with a very few number of sapling of *O. indicum* in nature. Keeping this in view, we have tried to germinate the seeds of this tree in nursery as well as, in laboratory conditions and found that seeds showed 48% germination in nursery and 96% in laboratory condition. To know the reason of cause of low germination in nursery condition we have tried to work out the seed mycoflora because certain mycoflora are distracting the seeds (Mehrotra, 1990; Ali and Nair, 1989). Hence, the present investigation was undertaken to find out whether the seed mycoflora is responsible for the sparse population of this tree in nature.

MATERIALS AND METHODS

Mature Seeds of *O. indicum* were collected at the time of peak seed fall from the Indian Central Himalaya (29° 43' N latitude and 79° 56'

*Corresponding author. E-mail: b. pande@yahoo.com.

Table 1. Effect of different experiments on germination percentage and different germination parameters.

Media	Germination percentage	Germination relative index	Days of emergence	Days of Completion	Germination index	Speed of Emergence	Mean emergence time
Blotting paper	96	163.2	4	13	28.8	15.5	6.83
PDA medium	20	24	10	18	6	1.72	14
Unsterilized nursery soil	0	0	0	0	0	0	0
Unsterilized habitat soil	0	0	0	0	0	0	0
Unsterilized sand	0	0	0	0	0	0	0
Sterilized nursery soil	40	20	11	25	12	2.35	18.25
Sterilized habitat soil	50	55	8	19	15	5.14	12
Sterilized sand	70	98	8	16	21	8.96	9.29

E longitude, brought to the laboratory and stored at room temperature for different experiments. Seed mycoflora of *O. indicum* was studied by using agar-plate method. The seeds were surface sterilized with 0.01% mercuric chloride solution followed by four times washing in sterilized distilled water. The seed germination behavior was studied in different media such as blotting paper, PDA medium, sterilized and unsterilized nursery soil (1:2:1), habitat soil and sand. Two layers of sterilized blotter papers soaked in sterilized distilled water were kept in Petri dishes on which the surface sterilized seeds were spread uniformly. In agar-plate method, sterilized petri plates containing PDA medium were employed. All the plates (containing blotting paper, PDA medium, sterilized and unsterilized nursery soil (1:2:1), habitat soil and sand) were incubated at 25±1^{o}C. Seed mycoflora of *Orox indicum* was identified on the basis of growth, mycelium, conidiophores and conidial morphology (Gilman, 2001; Barnett and Hunter, 1972; Chowdhry, 2000).

RESULTS

The seeds germinated in different media show different germination percentage (Table 1, Figure 1). It is revealed that the highest seed germination (96%) was recorded on blotting paper, followed by sterilized sand medium (70%), sterilized habitat soil (50%), sterilized nursery soil (40%) and on PDA medium (20%) (Figure 2). In case of unsterilized nursery soil, unsterilized habitat soil and unsterilized sand no germination took place. The germination was initiated on the 4th day after setting the experiment on blotting paper, on 8th day in sterilized habitat soil and sterilized sand, on 10th day on PDA medium and on 11th day in sterilized nursery soil. In case of unsterilized nursery soil, unsterilized habitat soil and unsterilized sand, the seeds were not germinated due to fungal attack. The germination relative index was found maximum in blotting paper (163.2), followed by sterilized sand medium (98), sterilized habitat soil (55), PDA medium (24) and sterilized nursery soil (20) (Figure 3A). The germination index followed the order: blotting paper (28.8) > sterilized sand medium (21) > sterilized habitat soil (15) > sterilized nursery soil (12) > PDA medium (6) (Figure 3B). The speed of emergence of seeds in different experiments followed the order: blotting paper (15.5) > sterilized sand (8.96) > sterilized habitat soil (5.14) > sterilized nursery soil (2.35) > PDA medium (1.72) (Figure 3C). The mean emergence time of seeds was found maximum in sterilized nursery soil (18.25), followed by PDA medium (14), sterilized habitat soil (12), sterilized sand (9.29) and blotting paper (6.83) (Figure 3D).

F. solani was isolated from the seeds in three experiments viz., unsterilized nursery soil, unsterilized habitat soil and unsterilized sand. Interestingly the same fungus was isolated from the seedling growing in the nursery (Figures 4A and B). The maximum number of fungi was observed on PDA medium and resulted in least germination percentage (20%). The mycoflora identified from the seeds, placed on PDA medium were *F. solani, A. solani, A.alternata, A. niger, A. nidulens, Trichoderma harzianum*, *C. lunata*., *S. chartarum, Acremonium* sp., *R. solani*, *Penicilium* sp., *Torula allii*, *C. globosum* and *C. cladosporioides*. This clarifies the high susceptibility of the seeds to fungi.

DISCUSSION

Several fungi that cause plant diseases are known to produce phytotoxins (Scheffer and Livingston, 1984; Janardhanan and Husain, 1980). A large number of seed-borne fungi produce toxic metabolites which often kill the embryo (Vidyasekaran et al., 1970). The fungus can synthesize proteolytic, pectinolytic and cellulolytic enzymes (Mathur and Gupta, 1981). In the present study, the germination of seeds may be inhibited due to the phytotoxins produced by the fungi. The fungi isolated in the present study might be producing a number of toxic metabolites (phytotoxins). The Phytotoxins produced by *F. solani* are two isomeric compounds marticin and isomarticin (Kern, 1972 vide Jin et al., 1996), javanicin (Baker et al., 1981 vide Jin et al., 1996) and a phytotoxic polypeptide monorden (Jin et al., 1996). Zearalenone is a field mycotoxin produced by *Fusaruim* spp. (Amadi and Adeniyi, 2009). *A. alternata* is also reported to produce tentoxin (cf. Templeton et al., 1967 vide Janardhanan, 1994), tenanzoic acid (Janardhanan, 1994), alternariol monoethyl ether (cf. Pero and Main, 1970 vide

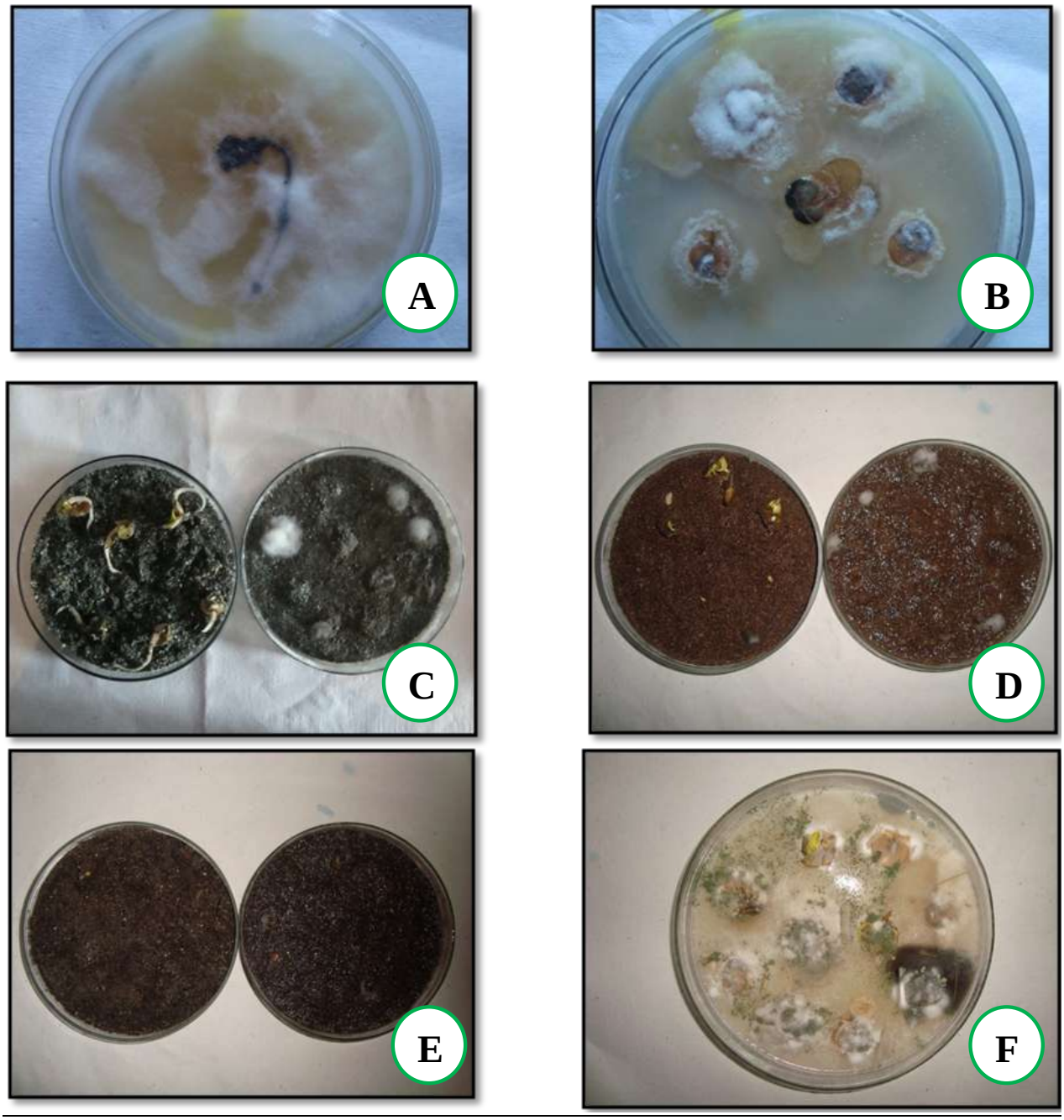

Figure 1. (A) Seedling colonized by fungi, (B) seeds colonized by fungi, (C) Seed germination in sterilized and unsterilized sand, (D) Seed germination in sterilized and unsterilized habitat soil, (E) Seed germination in sterilized and unsterilized nursery soil, (F) Seed germination in PDA medium.

Janardhanan, 1994), alternariols, tenuazonic acid (Xu et al., 2003). *A. solani* produce phytotoxins solanapyrones D and E (Oikawa et al., 1998). *A. niger* is reported to produce nigerazines (cf. Iwamoto et al., 1983, 1985 vide Cynthia, 2004), nigragillin (Caesar et al., 1969 vide Cynthia, 2004) and ochratoxins (Xu et al., 2003). *Trichoderma* sp. is reported to produce some lyophilized and non lyophilized toxins, 1,2-benzenedicarboxylic (Eziashi et al., 2010) while a *Penicillium* sp. is reported to produces ochratoxins (Xu et al., 2003).

From the data obtained it may be concluded that seeds and seedlings of *Oroxylum indicum* (L.) are highly susceptible to fungal decay. In the present study fourteen different seed-borne fungi were isolated. It was interesting to observe that some fungi (*A. solani, A. alternata, A. niger, Aspergillus nidulens, Trichoderma*

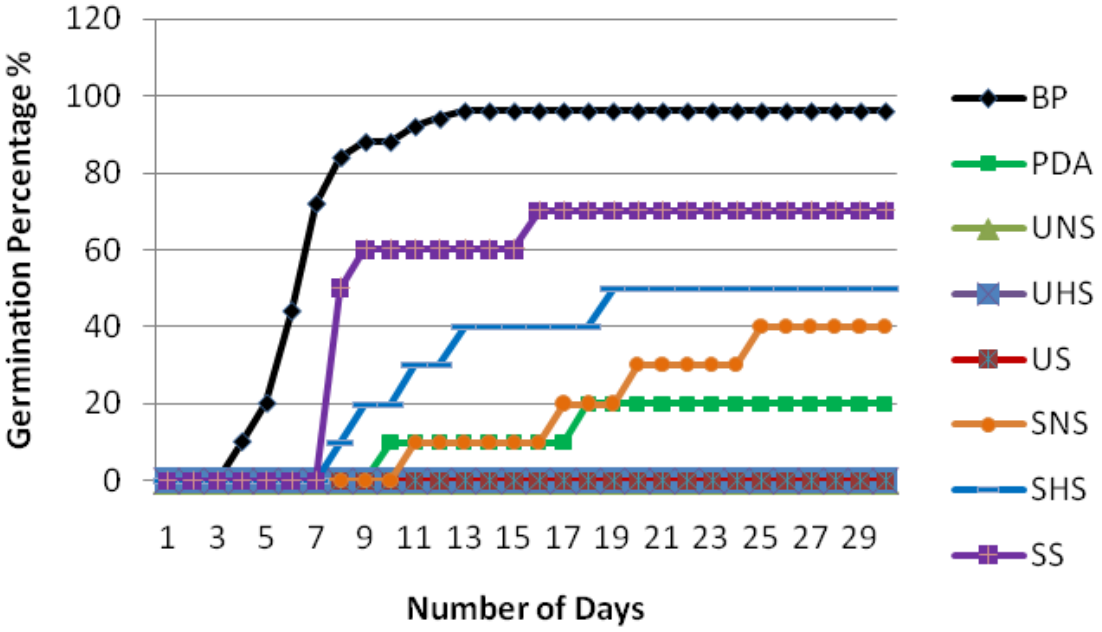

Figure 2. Showing seed germination in different media, Unsterilized nursery soil (UNS), Unsterilized habitat soil (UHS), Unsterilized sand (US), Sterilized nursery soil (SNS), Sterilized habitat soil (SHS), Sterilized sand (SS), Blotting paper (BP), Potato dextrose agar medium (PDA).

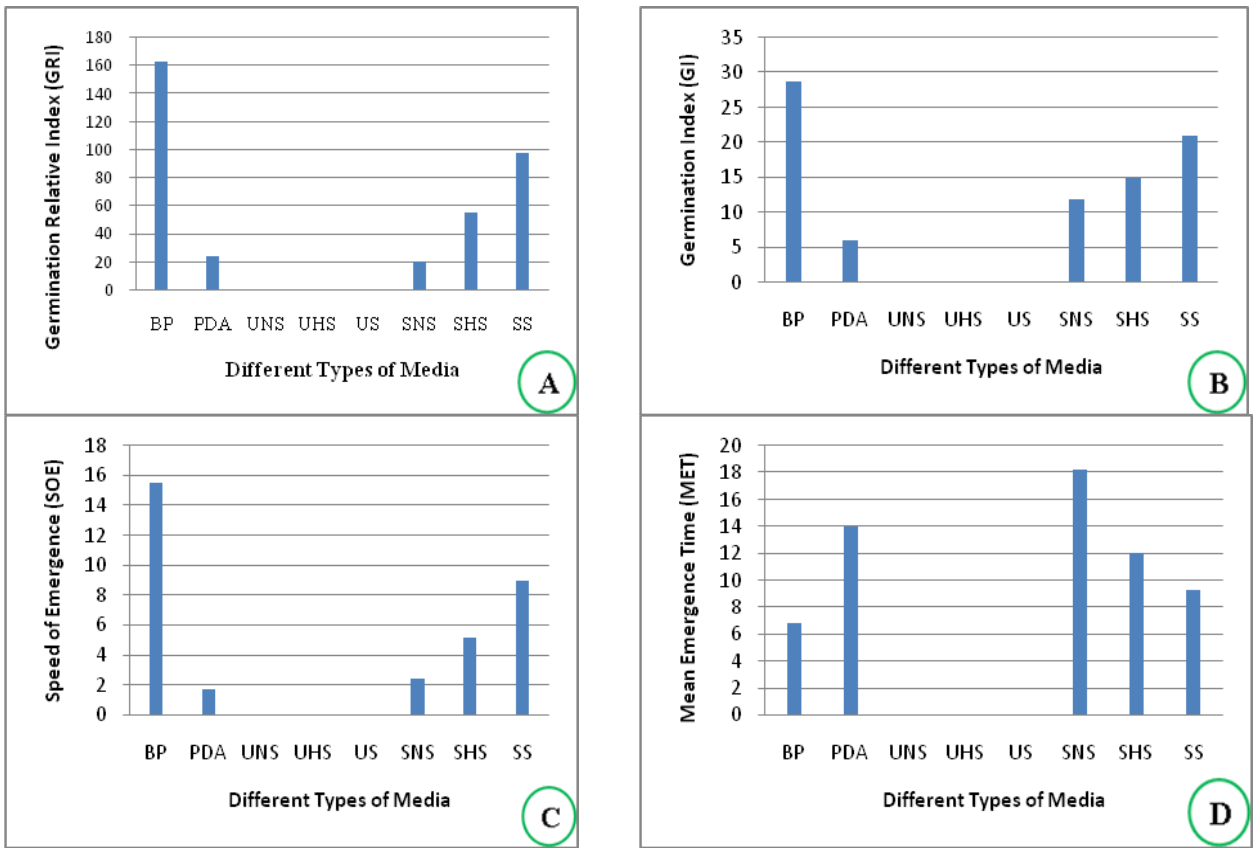

Figure 3. Showing effect of different experiments on seed germination parameters. (A) Germination Relative Index, (B) Germination index, (C) Speed of emergence, (D) Mean emergence time.

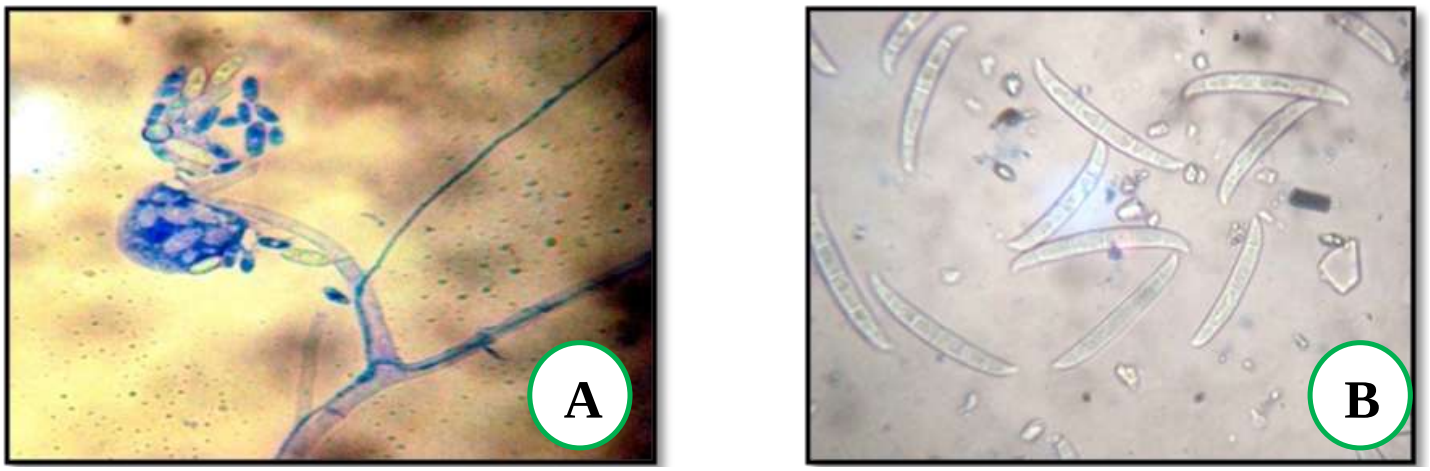

Figure 4. Photomicrograph of *Fusarium solani. (*A) Microconidia, (B) Macroconidia.

harzianum, *Curvularia lunata*, *Stachybotrys chartarum, Acremonium* sp., *Rhizoctonia solani*, *T. allii*, *C. globosum* and *C. cladosporioides*) were isolated from the seeds for the first time. Of these *Fusarium solani* was the most dominant which colonized and then damaged the seeds. The heavy mortality of seedlings in nursery condition due to damping off was caused by *F. solani.* It attacked the seedlings and the cotyledons turned black or yellow and rotten completely, resulting in the death of seedlings. Besides this post-emergence mortality, pre-emergence killing was also observed as indicated by the raised and split up soil at that place due to the upward pressure exerted by the raising seedlings and a large number of these seedlings also failed to emerge. This clearly explains the poor germination of seedlings in the nursery as compared to the germination obtained under laboratory conditions. Similar observations were also made by Mehrotra (1990). It is thus confirmed that the *Fusarium* is the main culprit for the destruction of the seeds. Many workers reported that *Fusarium* spp. causes scabby seed and damping off of seedlings (Castor and Fredericksen, 1980 vide Shetty and Prakash, 1994; cf. Gopinath, 1984 vide Shetty and Prakash, 1994). However, a species *Pythium intermedium* was also reported by Ali and Nair (1989) to cause damping off of the seedlings. This fungus is not recorded in the present study. Under natural conditions the seeds germinated early in the rainy season. The weather conditions during the seed germination are decisive for infection. It determines the extent of infection in seeds and the number of seeds infected. Hence, there is a need of management of seed deterioration for regeneration of this plant at large scale. Mehrotra (1990) suggested use of Ceresan for seed dressing to avoid the rotting of seeds in storage and to obtain better sprouting of seedlings of *O. indicum*. Further study is required to establish methods for maintenance, storage and protection of seedlings from microbes/fungi.

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