Indian Phytopath. 70 (2) : 206-215 (2017)
DOI 10.24838/ip.2017.v70.i2.70617

RESEARCH ARTICLE

Status of seed-borne fungi in some indigenous medicinal and aromatic plants conserved in National Gene Bank, India

JAMEEL AKHTAR[1]*, BALESHWAR SINGH[1], ARAVINDARAM KANDAN[1], PARDEEP KUMAR[1], ASHOK KUMAR MAURYA[1], DINESH CHAND[2], VEENA GUPTA[3] and SUNIL CHANDRA DUBEY[1]

[1]*Division of Plant Quarantine,* [3]*Division of Germplasm Conservation, ICAR-National Bureau of Plant Genetic Resources, New Delhi 110 012, India*

[2]*ICAR-National Bureau of Plant Genetic Resources, Regional Station, PDKV Campus, Akola 444 001, Maharashtra, India*

Received: 22 February 2017/ Accepted: 23 May 2017/ Published online: 25 May 2017

ABSTRACT: During 2011-2015, seed health testing (SHT) of 880 accessions representing more than 60 crop species of medicinal and aromatic plants (MAPs) germplasm resulted in detection and identification of 17 fungal species belonging to 11 genera. Based on morphological key characteristics, various pathogens, namely *Botrytis cinerea*, *Cephalosporium maydis, Colletotrichum gloeosporioides*, *Fusarium equiseti*, *F. oxysporum*, *F. semitectum*, *F. solani*, *F. verticillioides*, *Lasiodiplodia theobromae*, *Macrophomina phaseolina*, *Melanospora zamiae*, *Myrothecium roridum*, *M. verrucaria*, *Phoma exigua* var. *exigua*, *P. sorghina*, *Ustilago coicis* and *Verticillium albo-atrum* were recorded on 71 accessions (8.1%) of MAPs germplasm with varying level of seed infections (10-50%). Pathogen wise overall infection showed the highest infection share of *U. coicis* (44.4%) followed by *P. sorghina* (19.4%) and *B. cinerea* (11.1%) among 71 infected accessions. A perusal of literature indicated that detection of *B. cinerea* and *M. roridum* on *Vernonia anthelmintica*; *C. maydis* and *M. verrucaria* on *Costus speciosus*; *C. gloeosporioides* and *M. phaseolina* on *Abroma augusta*; *F. equiseti* on *Origanum vulgare*; *F. verticillioides* on *C. speciosus*, *Mucuna pruriens* and *Trichosanthes cucumerina*; *L. theobromae* on *C. speciosus*, *T.cucumerina* and *T. bracteata*; *P. exigua* var. *exigua* on *T. cucumerina*; and *P. sorghina* on *Coix lacryma-jobi, C. speciosus, Ochna lanceolata, Perilla frutescens*, *Tagetus patula*, *T. cucumerina* and *T. bracteata* are new host records on MAPs germplasm from India. If such infected seeds are conserved and/or distributed for either research purpose or their commercial use, they may act as a source of inocula dissemination and hamper the cultivation of MAPs leading to losses in quality and yield. Therefore, detection of seed infection through seed health testing is important in conserving disease-free material so as to minimize the risk of spreading disease in the country.

Keywords: Conservation, MAPs germplasm, seed health testing, seed-borne fungi

India has been considered as a treasure house of valuable MAPs/flora which constitutes an important natural wealth of our country as they play a significant role in providing primary health care services to the people. MAPs find a prime place in healthcare from ancient Indian Ayurveda, Siddha and Unani medical systems along with other schemes like homeopathic and allopathic systems (Gurib-Fakim, 2006). It is expected that sale of herbal medicines is likely to increase up to $ US 3 trillion by 2020 (Schippmann *et al*., 2002). The increased global demand for MAPs by various processing industries like nutraceuticals, pharmaceutical, cosmetic, food, etc. have resulted in placing pressure on natural resources, since most species used are still collected from their wild habitat (Gupta *et al*., 2013). Due to climate change and uncontrolled over-exploitation of wild plants, there is habitat loss of MAPs, that's why their evaluation, utilization and conservation have become essential to fulfilling the future demand (Barata *et al*., 2016). Therefore, ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India is undertaking exploration for indigenous MAPs germplasm collection with the aim of their safe conservation after SHT and utilization by providing germplasm worldwide.

*Corresponding author: jameel.akhtar@icar.gov.in

Nutrient-rich seeds are a good nutritional substrate for the growth of various microorganisms, including pathogens. Pathogens which damaging the root, leaf and stem are transmitted through seeds or which inhabit the soil environment, decrease the aesthetic values of ornamentals crops (Gilbert, 2003; Kiecana *et al*., 2012). Infected seed is the primary source of inoculum for infection in seedlings.

Medicinal plants are also attacked by fungi, bacteria, viruses, phytoplasma/mollicutes, nematodes and some are notably notorious and lead to epidemics under favourable environment. Little attention towards the cause and their control has been given so far (Sinha *et al*., 2002). Various pathogens adversely affect the medicinal plant parts and decrease in the medicinal value. It may be harmful to the human body while using these infected plant parts as medicine (Chavan and Korekar, 2011). Review of literature reveals that pathogenic as well as saprophytic fungi predominantly colonize MAPs seeds (Raut *et al.*, 2016). The most common saprophytic fungi colonizing seeds comprises *Alternaria*, *Cladosporium*, *Rhizopus*, *Mucor*, *Aspergillus* and *Penicillium*. Pathogenic fungi namely, *Fusarium* spp., *Lasiodiplodia theobromae* and *Ustilago coicis* etc. needs more attention among pathogenic fungi colonizing seeds because they produce toxins and enzymes which play an important role in the pathogenesis (Raut *et al.,* 2016; Richardson, 1990; Titatarn *et al*., 1983). However, scanty information is available on pathogens of MAPs in the literature (Dev *et al*., 2010). Therefore, keeping in view the importance of MAPs germplasm, study was undertaken to assess their seed health status for their safe conservation in the National Gene Bank for future.

MATERIALS AND METHODS

The seeds of 880 accessions (Table 1) representing more than 60 genera of indigenously multiplied/collected MAPs germplasm from different agro-ecological zones of the country were received for SHT during 2011-2015. Seed health testing of MAPs germplasm was conducted in the Division of Plant Quarantine, ICAR-National Bureau of Plant Genetic Resources, New Delhi, India to detect and identify the fungal pathogens associated with them before conservation in the National Gene Bank. The seed samples of all the accessions were first examined visually and then abnormal, discoloured and deformed seeds etc. were subjected to blotter test (Mathur and Kongsdal, 2003).

The seeds were surface sterilized by immersing in 4.0% NaOCl for 30 seconds, subsequent rinsing three times in sterilized distilled water under aspetic conditions using laminar air flow. Depending upon availability and size of seeds, 10-25 seeds per Petri plate (110 mm) were placed at equidistance on three layers of sterilized moist blotters and labelled. The seeded plates were then incubated for 7 days at 22±1°C under alternating cycles of 12 h light and darkness. On 8th day, the incubated seeds were observed under stereomicroscope (Nikon-SMZ 1500) at different levels of magnification ranging from 0.75x to 11.25x for detection of fungal

Table 1. List of medicinal and aromatic plants germplasm tested for seed-borne fungal pathogens.

Common name	Botanical name	Common name	Botanical name
Devil's cotton	*Abroma agusta* (4)*	Mallotus	*Mallotus philippensis* (1)
Indian liquorice	*Abrus precatorius* (3)	Mallow	*Malva* spp. (3)
Bead Tree	*Adenanthera* spp. (3)	Ice Plant	*Martynia diandra* (1)
King of bitters	*Andrographis paniculata* (7)	Touch-me-not	*Mimosa pudica* (1)
Indian birthwort	*Aristolochia indica* (1)	Four o'clock Plant	*Mirabilis jalapa* (1)
Vetches	*Asparagus racemosus* (8)	Noni	*Morinda* spp. (66)
Annato	*Bixa orellana* (2)	Drumstick	*Moringa oleifera* (1)
Shivlingi	*Bryonopsis laciniosa* (1)	Kewanch	*Mucuna* spp. (7)
Callicarpa	*Callicarpa macrophylla* (1)	Katharai	*Ochna lanceolata* (4)
Bhang	*Cannabis sativa* (1)	Basil	*Ocimum* spp. (80)
Bittercress	*Cardamine oligosperma* (1)	Oregano	*Origanum vulgare* (32)
Caraway	*Carum* spp. (14)	Broken bones plant	*Oroxylum indicum* (2)
Jyotismati	*Celasrtus paniculatus* (2)	Amrul	*Oxalis corniculata* (1)
Centratherum	*Centratherum anthelminticum* (1)	Ranghevada	*Paracalyx scariosa* (1)
Safed Musli	*Chlorophytum borivilianum* (1)	Perilla	*Perilla frutescens* (4)
Spider flower	*Cleome viscose* (7)	Aonla	*Phyllanthus emblica* (1)
Glorybower	*Clerodendron viscosum* (1)	Isabgol	*Plantago ovate* (28)
Tear grass	*Coix lacryma-jobi* (402)	Common purslane	*Portulaca oleracea* (1)
Kemuk	*Costus speciosus* (6)	Round galangal	*Premna herbacea* (1)
Palmarosa grass	*Cymbopogon martini* (3)	Raspberry	*Rubus ellipticus* (1)
Datura	*Datura* spp. (3)	Sage/ chia	*Salvia hispanica* (2)
Persimmon tree	*Diospyros melanoxylon* (1)	Soapberry	*Sapindus mukorossi* (1)
Malaysian apple	*Diplocyclos palmatus* (3)	Chirayata	*Swertia* spp. (6)
False black pepper	*Embelia ribes* (1)	Tephrosia	*Tephrosia purpurea* (3)
Brilliant gardenia	*Gardenia resinifera* (1)	Myrobalan	*Terminalia* spp. (2)
Henbane	*Hyoscyamus niger* (1)	Ban Kapas	*Thespesia lampas* (1)
Indigofera	*Indigofera tinctoria* (1)	Desert horse purslane	*Trianthema portulacastrum* (3)
Lagerstroemia	*Lagerstroemia parviflora* (2)	Snake gourd	*Trichosanthes* spp. (107)
Leea	*Leea macrophylla* (1)	Kalijiri	*Vernonia anthelmintica* (5)
Leucaena	*Leucaena leucocephala* (1)	Ashwagandha	*Withania somnifera* (28)

*Values in parentheses show number of accessions.

fructification(s) associated with seeds, if any. The slides were also prepared by mounting with lactophenol cotton blue stain for identification of fungus and observed under compound microscope (Nikon - Eclipse 80i). Further, to establish the identity of causal fungus, pure culture of the associated fungus was obtained on potato dextrose agar (PDA) by transferring its single spore grown on seeds (Akhtar *et al*., 2014). The observations on frequency of fungal infection and seed germination were recorded and correlation analysis was done. DIVA-GIS 7.5.0 software was used to point data into grid analysis using simple-circular neighborhood methods (Hijmans *et al.*, 2001) and diversity was calculated using Shannon-Wiener diversity index (Magurran, 1988).

RESULTS AND DISCUSSION

Visual examination of SHT ensured detection of *Ustilago coicis* Bref*.,* a smut fungus, in 32 accessions of *Coix lacryma-jobi* (IC-006645, IC-006667, IC-012637, IC-012639, IC-012703, IC-089381, IC-089382, IC-089383, IC-089384, IC-089387, IC-089389, IC-089391, IC-089392, IC-204184, IC-340015, IC-374506, IC-416971, IC-418897, IC-521338, IC-521339, IC-521341, IC-521343, IC-540173, IC-540181, IC-540222, IC-540244, IC-540256, IC-540266, IC-540267, IC-540279) collected from Shillong, Meghalaya and RS/RS-I-20-2011 and RS/RS-I-32-2011 from New Delhi. This disease is reported to cause yield loss of 12-25% and is widespread in Assam (Choudhary, 1946). Titatarn *et al*. (1983) also reported yield loss due to *U. coicis* ranging from 15-98% in Thailand.

After 7 days of incubation, critical examination of MAPs germplasm seeds under the stereozoom microscope at different levels of magnification resulted in detection and identification of 16 species belonging to 10 fungal genera of economic importance. Based on the growth characteristic and fruiting structures observed on seed/seedlings as described by Mathur and Kongsdal (2003) and IMI descriptions for fungi and bacteria, the associated fungi were identified as *Botrytis cinerea* Pers. Fr., *Cephalosporium maydis* Samra, Sabet & Hingorani, *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., *Fusarium equiseti* (Corda) Sacc. (tel.: *Gibberella intricans* Wollenw.), *F. oxysporum* Schlechtendahl, *F. semitectum* Berk. & Rav., *F. solani* (Martius) Sacc., *F. verticillioides* (Sacc.) Nirenberg (syn.: *F. moniliforme* Sheldon), *Lasiodiplodia theobromae* (Pat.) Griffiths & Maubl. (syn.: *Botryodiplodia theobromae*), *Macrophomina phaseolina* (Tassi) Goid (syn.: *Rhizoctonia bataticola*), *Melanospora zamial* Corda, *Myrothecium roridum* Tode ex Fr., *M. verrucaria*, *Phoma exigua* var. *exigua* Desm. (syn.: *P. herbarum*), *P. sorghina* (Sacc.) Boerema Dorenbosch & van Kest. (syn.: *P. insidiosa*), *Verticillium albo-atrum* Reinke & Berthold in 39 accessions of 12 species belonging to 10 MAPs genera (Table 2).

Botrytis cinerea, a phytopathogenic fungus, was detected on seeds of three accessions of *Costus speciosus* from Bokaro (IC-439154) and Giridih (IC-587520) of Jharkhand and Purulia (IC-336849) of West Bengal, in *Mucuna pruriens* (IC-613962) from Valsad, Gujarat, *Ocimum sanctum* (IC-612500) from Ganjam, Odisha and *Vernonia anthelmintica* (IC-587521) (Fig. 1a) from Ranchi, Jharkhand and on two accessions of *Perilla frutescens* from Ukhrul, Manipur (IC-615362) and Kohima, Nagaland (KCB3) with varying level of infection ranging from 10-20% (Table 3). It causes botrytis rot and gray mould in over 200 plant species of economic importance including grain legumes (Pande *et al.,* 2006). Seed germination was also affected by this fungus which ranged from 0-90%. It is very difficult to assess the damage caused by *B. cinerea*. However, economic losses of >50% may occur in many crops, depending on the prevailing environmental conditions (CAB International, 2007).

Cephalosporium maydis, a fungus causing black bundle disease/late wilt of maize, seed rot, etc., was detected in one accession (IC-587520) of *Costus speciosus* from Jharkhand with 20% infection, which is internally and externally seed-borne pathogen on limited host comprising maize, cotton and lupins with limited geographical distribution including India. Its widespread incidence and severity of 100% infection have been reported in some fields (Samra *et al*., 1963).

Colletotrichum gloeosporioides, a causal agent of anthracnose/ fruit rot, was detected (10% infection) in only one accession (IC-331486) of *Abroma augusta* (Fig. 1c) from Kolkata, West Bengal. This fungus causes a highly destructive anthracnose/ fruit-rot disease on a wide variety of monocotyledonous plants to higher dicotyledonous trees (Nelson, 2008). It causes considerable damage to a large number of crops such as cereals, legumes, and tropical, subtropical fruit crops including banana, coffee mango, etc., (Sharma and Kulshrestha, 2015).

Fusarium equiseti, the causal organism of damping-off, stalk rot, post-harvest decay, etc., was detected and identified with 40% infection from seeds of one accession (IC-589099) of *Origanum vulgare* (Fig. 1b) from Uttarakhand. *F. equiseti* is associated with more than 50 crops species including *Helianthus annuus*, *Brassica oleracea* var*. capitata, Capsicum annum, Cuminum cyminum*, *Glycine max, Oryza sativa, Solanum lycopersicum, S. tuberosum, Sorghum bicolor, Triticum aestivum, Vicia faba* and *Zea mays,* etc., across the world (CAB International, 2007). The fungus is considered as a weak parasite. However, it could cause serious damage and high losses between 10-45% to seedling in commercial nurseries.

Fusarium oxysporum, the destructive wilt pathogen was detected with 100% infection level in two samples *viz*., KP/BA-1976 and KP/BA-2004 of *Trichosanthes bracteata* collected from Visakhapatnam and West Godavari, Andhra Pradesh, respectively. Losses caused by this fungus is not available in the literature. However, Janardhan *et al.* (1964) reported Fusarium wilt due to *Fusarium oxysporum* f. sp. *rauvolfiae* from Jammu. Medicinal plant species of *Swertia chirayita, Rauvolfia*

Table 2. Morphological features of fungi detected in medicinal and aromatic plants germplasm

Fungus	Growth characteristics			
	Shape/type	Septation	Dimension	Attachment
Botrytis cinerea	Conidiophores with characteristic 'twisting' at several places	Single-celled with slight protuberant hilum	Length 8.0-14.0 µm and width 6-9 µm	Ashgrey to greyish clusters of conidia at several places of conidiophores
Cephalosporium maydis	Conidia hyaline, ellipsoidal to cylindrical, straight with rounded ends	Conidia single-celled	Length 3.0-10.0 µm and width 1.5-3.0 µm	Conidia in the form of shiny, round and watery heads attached perpendicular to conidiophores
Colletotrichum gloeosporioides	Acervuli sub-epidermal emerging by disrupting outer epidermis of the seed	Setae dark brown, rigid, swollen at the base, slightly tapered to the paler acute apex, 1 to 5-septate, 250 x 6 µm	Conidial length 18.0-23.0 and width 3.0-5.0 µm	Conidia hyaline, with narrow truncate base, aseptate, uninucleate
Fusarium equiseti	Macroconidia hyaline, falcate with pedicellate foot cell attaining apical cell inward	Macroconidia mostly non septate and macroconidia mostly 5 septate, but sometimes 3 to 5-septate	Macroconidia 22.0-60.0 µm long and 3.0-6.0 µm wide	Macroconidia produced in creamy or organge coloured slimy mass, called as 'pinnotes' on seed
Fusarium oxysporum	Microconidia oval, elliptical or reniform and macro-conidia falcate along with chlamydospores	Microconidia mostly non septate and macroconidia mostly 3-septate, sometimes 3-5 septate	Microconidia 5-12 µm long and 2-3.5 µm wide; macroconidia mostly 27-65 µm long and 3-5 µm wide	Microconidia formed in false-heads on monophialides macroconidia in slimy mass
Fusarium semitectum	Macroconidia hyaline, straight to slightly curved, wedg-shaped without pedicellate basal cell	Macroconidia mostly 3-5 septate	Macroconidia 17.0-40.0 µm long and 2.0-4.0 µm wide	Branch conidiophores/ phialides bear single macroconidia, sometimes conidia in pinnotes giving flower like structure.
Fusarium solani	Microconidia hyaline, oval, ellipsoidal or reniform and macroconidia thick-walled, hyaline with short rounded apical cell	Microconidia mostly 0-1 septate and macro-conidia mostly 3-4 septate	Microconidia 8-15 µm long and 2-4 µm wide, macroconidia mostly 45.0-100 µm long and 5-8 µm wide	Microconidia formed in translucent to opaque, milky white watery drops on long phialides; macroconidia produced in cream coloured sporodochia
Fusarium verticillioides	Microconidia mostly crescent shaped and macroconidia fusoid with sharply curved pedicellate basal cell	Microconidia occasionally one-septate and macro-conidia mostly 3 septate	Microconidial length 5-12 µm and width1.5-2.5 µm, macroconidia mostly 25-60.0 µm long and 2.5-4 µm width	Microconidia in chains and macroconidia in wet mass
Lasiodiplodia theobromae	Pycnidia brown to dark brown	Non-septate, hyaline, sometimes guttulate	Conidial dimension ranged between 3.5-4.5 x 1.5 µm	Pycnidia erumpent with silvery white mycelium
Macrophomina phaseolina	Dark brown to black coloured large pycnidia grown with mycelium surrounding the neck of the pycnidia	Spores non-septate, hyaline, ellipsoide to obovoid	Spore size measured 15.0-30.0 x 5.0-10.0 µm	Spores oozing from osteole in white cooured cirrhus
Melanospora zamia	Perithecia produced superficially on seed surface with leon-shaped ascospore oozing from neck	Single-celled citriform and dark brown to blackish ascospores	Conidial dimension measured between 15.0-25.0 x 11-15 µm	Spores oozing from the neck in black cooured long-very long cirrhus
Myrothecium roridum	Conidia hyaline to light green and cylindrical with slightly rounded end	Conidia single-celled	Conidial size measured 5.0-7.0 x 1.0-2.0 µm	Spores formed in blackish green to black sporodochia on seed surface
Myrothecium verrucaria	Conidia hyaline to olive green, elliptical with pointed end	Conidia single-celled	Conidial size measured 5.0-7.0 x 2.5-3.5 µm	Spores formed in irregular-shaped, green to dark green, flat sporodochia on seed surface
Phoma exigua var. *exigua*	Pycnidia brown to black, slightly embedded in seed coat	Conidia non-septate, hyaline, mostly straight and guttulate, cylindrical	Conidial dimension ranged between 5.0-10.0 x 2.5-3.0 µm	Conidia produced in pycnidia embedded in seed coat

Contd...

Fungus	Growth characteristics			
	Shape/ type	Septation	Dimension	Attachment
Phoma sorghina	Profuse mycelium, pycnidia black and shiny with small to long neck	Non-septate, hyaline, cylindrical and guttulate conidia	Conidial dimension ranged between 3-7 x 2-2.5 µm	Conidia produced in superficially developed pycnidia on aerial mycelium
Ustilago coicis	Mass of teliospores of dark brown colour, sub-globose to ellipsoidal in shape with minute echinulations giving the margin a serrated appearance	Teliospore germinates into 4-celled promycelium.	The teliospores dimension ranged between 7.0-14.0 µm in diameter	Sporidia formed terminally as well as laterally near the septa
Verticillium albo-atrum	Verticils of phialieds bearing small colourless, circular, shiny watery drops at the tips of each vertical	Single-celled, occasionally 1-septate	Conidial dimension measured 3.5-10.5 µm in length and 2.0-4.0 µm in width	Verticils of phialides arranged erect on conidiophores

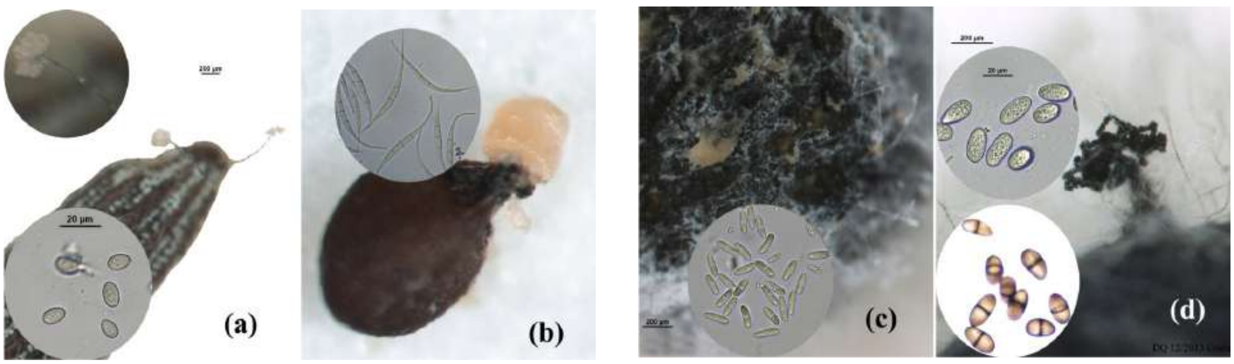

Fig. 1. Important seed-borne fungi detected on medicinal and aromatic crops; (a) *Botrytis cinerea* on *Vernonia anthelmintica,* (b) *Fusarium equiseti on Origanum vulgare*, (c) *Colletotrichum gloeosporioides* on *Abroma augusta* and (d)*, Lasiodiplodia theobromae* on *Costus speciosus*

serpentina, Lavandula angustifolia and *Aloe barbadensis* have also been reported severely infected with wilt from Himachal Pradesh, India by Chandel *et al.* (2014).

Fusarium semitectum, the causal agent of diseases like wilts, blights, root rots, and cankers in coffee, pine trees, wheat, corn, rice, cereals, carnations and grasses was detected in three accessions of *Costus speciosus* (IC-336849 from Purulia, West Bengal; IC-439154 from Bokaro and IC-587520 from Giridih, Jharkhand) and in one accessions of *Coix lacryma-jobi* (IC-435150) from Dumka, Jharkhand with varying infection level ranged between 70-90%. The pathogen is both externally and internally seed-borne. The internal inoculum affects seed germination, viability and caused high losses (pre- and post-emergence) from 15 to 75%. Gupta *et al.* (2011) have reported heavy losses to the *Shisham* tree plantation due to its transmission from seed to seedling. Ingle and Rai (2011) have reported genetic diversity among Indian phytopathogenic isolates of *F. semitectum.*

Fusarium solani, causes wilt and damping-off disease on a number of crop species, was detected in two accessions of *Costus speciosus* (IC-336849 from Purulia, West Bengal and IC-587520 from Giridih, Jharkhand) and two accessions of *Trichosanthes cucumerina* (IC-618003 from Udham Singh Nagar, Uttrakhand and IC-596997 from Changlang, Arunachal Pradesh) with infection level of 10-20%. *F. solani* causes substantial economic losses worldwide including wilt of *Lavandula angustifolia* in India (Chandel *et al*., 2014). Moreover, molecular studies revealed a high level of diversity within the fungal population (Saremi *et al.,* 2011).

Fusarium verticillioides, the causal organism of bakane/stalk/stem/ear rots diseases, was detected in one accession each of *Coix lacryma-jobi* (IC-089385) from Shillong, Meghalaya and *Costus speciosus* (IC-587520) from Giridih, Jharkhand and two accessions each of *Mucuna pruriens* (SKMP-15 from Rangpoh, Sikkim and RJSSMP-07 from Jhalawar, Rajasthan) and *T. cucumerina* (KP/NSP1430 and KP/NSP1441) from Udham Singh Nagar, Uttrakhand with average infection level of 10-30%. This fungus is reported to cause huge yield losses up to 75% in rice from Iran (Saremi *et al.*, 2008) and also has genetic variability (Sharma *et al.,* 2014).

Lasiodiplodia theobromae, a plurivorous and secondary pathogen, was detected during incubation test in one accession each of *Costus speciosus* (IC-439154) from Bokaro, Jharkhand (Fig. 1d), *Tricosanthes bracteata* (KP/BA-2018) from West Godavari, Andhra Pradesh and *Trichosanthes cucumerina* (IC-618003) from Udham Singh Nagar, Uttrakhand and in three accessions of

Table 3. Important seed-borne fungi detected on seeds of some indigenous medicinal and aromatic plants germplasm

Pathogen	Crop	Accession/ infection (%)	Source
Botrytis cinerea	*Costus speciosus*	IC-439154 (20%) IC-336849 (10%) IC-587520 (10%)	Bokaro, Jharkhand Purulia, West Bengal Giridih, Jharkhand
	Mucuna pruriens	IC-613962 (20%)	Valsad, Gujarat
	Ocimum sanctum	IC-612500 (10%)	Ganjam, Odisha
	Perilla frutescens	IC-615362 (10%) KCB3 (10%)	Ukhrul, Manipur Kohima, Nagaland
	Vernonia anthelmintica	IC-587521 (30%)	Ranchi, Jharkhand
Cephalosporium maydis	*C. speciosus*	IC-587520 (20%)	Giridih, Jharkhand
Colletotrichum gloeosporioides	*Abroma augusta*	IC-331486 (10%)	Kolkata,West Bengal
Fusarium equiseti	*Origanum vulgare*	IC-589099 (40%)	Pauri, Uttrakhand
F. oxysporum	*Trichosanthes bracteata*	KP/BA-1976 (100%) KP/BA-2004 (100%)	Visakhapatnam, Andhra Pradesh West Godavari, Andhra Pradesh
F. semitectum	*C. speciosus*	IC-336849 (70%) IC-439154 (80%) IC-587520 (90%)	Purulia, West Bengal Bokaro, Jharkhand Giridih, Jharkhand
	Coix lacryma-jobi	IC-435150 (80%)	Dumka, Jharkhand
F. solani	*C. speciosus*	IC-336849 (20%) IC-587520 (10%)	Purulia, West Bengal Giridih, Jharkhand
	Trichosanthes cucumerina	IC-618003 (20%) IC-596997 (10%)	Udham Singh Nagar, Uttrakhand Changlang, Arunachal Pradesh
F. verticillioides	*C. lacryma-jobi*	IC-089385 (10%)	Shillong, Meghalaya
	C. speciosus	IC-587520 (20%)	Giridih, Jharkhand
	M. pruriens	SKMP-15 (20%)	Rangpoh, Sikkim
		RJSSMP-07 (30%)	Jhalawar, Rajathan
	T. cucumerina	KP/NSP1430 (20%), KP/NSP1441 (10%)	Udham Singh Nagar, Uttrakhand
Lasiodiplodia theobromae	*C. speciosus*	IC-439154 (10%)	Bokaro, Jharkhand
	T. bracteata	KP/BA-2018 (100%)	West Godavari, Andhra Pradesh
	T. cucumerina	IC-618003 (40%)	Udham Singh Nagar, Uttrakhand
	Trichosanthes sp.	IC-539383, IC-539384, IC-539386 (100% each)	Nainital, Uttrakhand
Macrophomina phaseolina	*A. augusta*	IC-557342 (30%)	Kolkata, West Bengal
Melanospora zamiae	*C. speciosus*	IC-587520 (20%)	Giridih, Jharkhand
Myrothecium roridum	*V. anthelmintica*	IC-572783 (10%) IC-587521 (20%)	Solan, Himachal Pradesh Ranchi, Jharkhand
M. verrucaria	*C. speciosus*	IC-439154 (10%)	Bokaro, Jharkhand
Phoma exigua var. *exigua*	*T. cucumerina*	KP/NSP1430 (40%)	Udham Singh Nagar, Uttrakhand
P. sorghina	*C. lacryma-jobi*	IC-435150 (40%)	Dumka, Jharkhand
		IC-089385 and IC-416826 (10% each), IC-524661, IC-540267 (20% each)	Shillong, Meghalaya
	C. speciosus	IC-439154 (20%) IC-587520 (20%)	Bokaro, Jharkhand Giridih, Jharkhand
	Ochna lanceolata	IC-373679 (10%)	Kottayam, Kerala
	P. frutescens	RS/RS-I-2-2011 (10%) SHL1316 (50%)	Kohima, Nagaland Ukhrul, Manipur
	Tagetes patula	IC-318942 (30%)	Nainital, Uttrakhand
	T. cucumerina	DPP-10-41 (30%)	Boudh, Odisha
	T. bracteata	IC-539383, IC-539385 (100% each)	Nainital, Uttrakhand
Verticllium albo-atrum	*T. bracteata*	KP/BA-1977 (10%)	Vizianagaram, Andhra Pradesh
	M. pruriens	SKMP-15 (20%)	Rangpoh, Sikkim
		IC-615378 (10%)	Ukhrul, Manipur

Trichosanthes sp. (IC-539383, IC-539384 and IC-539386) from Nainital, Uttrakhand with infection level of 10-100%. It has been found to affect a wide range of hosts including more than 40 host crops causing numerous diseases such as fruit rot of coconut, leaf blight of *Pandanus odoratissimus*, the rot of cashew kernel, seedling mortality of maize, etc. (Punithalingam, 1980). In addition, seed rot and blackening of seeds has also been reported to affect seed germination, viability and vigour (Gure *et al.*, 2005, Nwachukwu and Umechuruba, 1996).

Macrophomina phaseolina, the causal agent of charcoal rot, ashy stem blight and root rot in a variety of crops, was detected in one accession (IC-557342) of *Abroma augusta* (Fig. 2a) from Kolkata, West Bengal with 30% infection level. A yield loss of 31-38% has been reported in sorghum from India due to this fungus (CAB International, 2007). Mahdizadeh *et al.* (2011) reported high levels of variability within *M. phaseolina*.

Melanospora zamiae, the causal agent of crown rust of maize was detected in only one accession (IC-587520) of *C. speciosus* from Giridih, Jharkhand. The report of the Technical Working Group 1 of Australia listed this as a pathogen of quarantine significance and highlighted the risk associated with the bulk maize import from USA (Irwin *et al.*, 1999).

Myrothecium roridum, a cosmopolitan plant pathogen with a broad host range causing blight, leaf spot, ring rot and stem necrosis, was detected with 10-20% infection level in two samples of *Vernonia anthelmintica* (Fig. 2b) from Ranchi, Jharkhand (IC-587521) and Solan, Himachal Pradesh (IC-572783). It infects a large number of plant hosts, including vegetables, fruits and ornamental plants (Hong *et al.*, 2013; Okunowo *et al.*, 2010).

Myrothecium verrucaria, a fungus causing leaf spot, blotch and blight in a various crop species, was detected in only one accession (IC-439154) of *Costus speciosus* from Jharkhand with infection level of 10%. *Mucuna verrucaria* has been reported to infect many hosts *viz.*, *Allium cepa, Arachis hypogaea, Avena sativa, Capsicum* spp*., Coffea* spp*., Gossypium* spp*., Morus* sp*., Nicotiana tabacum, Pennisetum glaucum, Phaseolus vulgaris,* etc. (Yang, 1995).

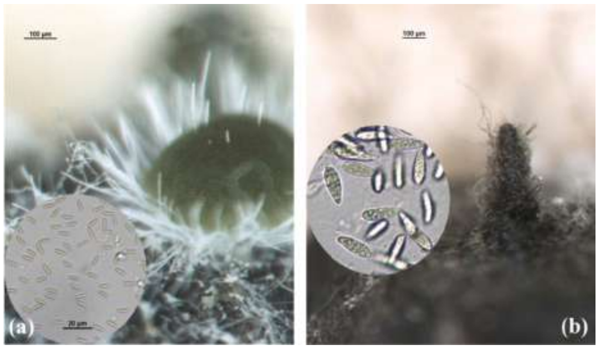

Fig. 2. *Macrophomina phaseolina* detected on *Abroma augusta* (a) and *Myrothecium roridum* on *Vernonia Anthelmintica* (b)

Phoma exigua var. *exigua*, a fungus causing leaf spot/leaf blotch/root rot/tuber rot, was detected in one accession of *Trichosanthes cucumerina* (KP/NSP1430) from Udham Singh Nagar, Uttarakhand with seed infection of 40%. This fungus is a ubiquitous weak or secondary pathogen, which infects more than 200 different plant genera worldwide (CAB International, 2007).

Phoma sorghina, a weak pathogen, causes leaf spot, glume blight in several grasses, was detected in 14 accessions of *C. lacryma-jobi* (IC-435150 from Dumka, Jharkhand, IC-089385, IC-416826, IC-524661 and IC-540267 from Shillong, Meghalaya), in *Costus speciosus* (IC-439154 from Bokaro, IC-587520 from Giridih, Jharkhand), in *Ochna lanceolata* (IC-373679 from Kottayam, Kerala), in *Perilla frutescens* (RS/RSI-2-2011 from Kohima, Nagaland, SHL1316 from Ukhrul, Manipur), in *Tagetus patula* (IC-318942 from Nainital, Uttrakhand) and in *T.* cucumerina (DPP-10-41 from Boudh, Odisha and *T. bracteata* (IC-539383 and IC-539385) from Nainital, Uttrakhand with varying degree of infection ranging from 10-100%. *P. sorghina* has a wide host range with worldwide distribution (Singh *et al.*, 2014). Yield losses up to 14% due to glume blight caused by *P. sorghina* in rice has been reported and the pathogen was earlier considered to be of minor economic importance but attained epidemic proportions over a large geographical area during 1979-80 (Prabhu and Bedendo, 1988).

Verticillium albo-atrum, a causal agent of verticillium wilt disease in several crops, was detected in *T. bracteata* (KP/BA-1977) from Vizianagaram, Andhra Pradesh and in *M. pruriens* (SKMP-15 from Rangpoh, Sikkim, and IC-615378 from Ukhrul, Manipur) with infection level of 10.0-20.0%). A yield loss of 10-15% in rockwool crop, with occasional dead plants; and 20-25% plant death has been reported in rockwool, hydrophonics and soil-grown crops (CAB International, 2007).

Keeping in view occurrence of the above-mentioned pathogens and resulting yield losses in crops other than MAPs as well as the existence of genetic variability in them, the detection and identification of 17 fungal species belonging to 11 genera in 12 species of medicinal and aromatic crops could be a potential threat in future for their cultivation.

As per perusal of literature, there is no record of *Botrytis cinerea* and *Myrothecium roridum* on *Vernonia anthelmintica*; *Cephalosporium maydis* and *Myrothecium verrucaria* on *Costus speciosus*; *Colletotrichum gloeosporioides* and *Macrophomina phaseolina* on *Abroma augusta*; *Fusarium equiseti* on *Origanum vulgare*; *Fusarium verticillioides* on *Costus speciosus*, *Mucuna pruriens* and *Trichosanthes cucumerina*; *Lasiodiplodia theobromae* on *Costus speciosus T. cucumerina* and *T. bracteata*; *Phoma exigua* var. *exigua* on *T. cucumerina* and *T. bracteata* and *Phoma sorghina*

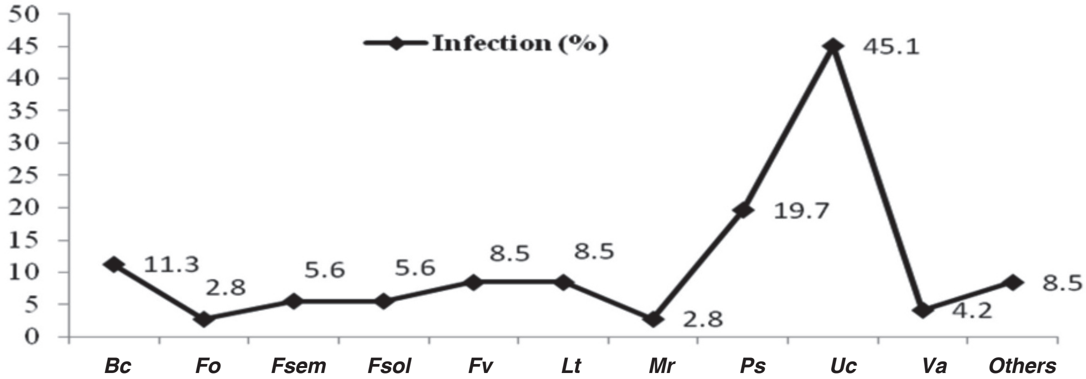

Fig. 3. Pathogen-wise fungal infection (%) detected in medicinal and aromatic plants germplasm. (*Bc= Botrytis cinerea*; *Cm= Cephalosporium maydis; Fo= Fusarium oxysporum*; *Fsem= F. semitectum*; *Fsol= F. solani*; *Fv= F. verticillioides*; *Lt= Lasiodiplodia theobromae*; *Mr= Myrothecium roridum*; *Ps= Phoma sorghina*; *Va= Verticillium albo-atrum*). Other pathogens include *Colletotrichum gloeosporioides*, *Fusarium equiseti*, *Macrophomina phaseolina*, *Melanospora zamiae*, *Myrothecium Verrucaria* and *Phoma exigua* var. *exigua*

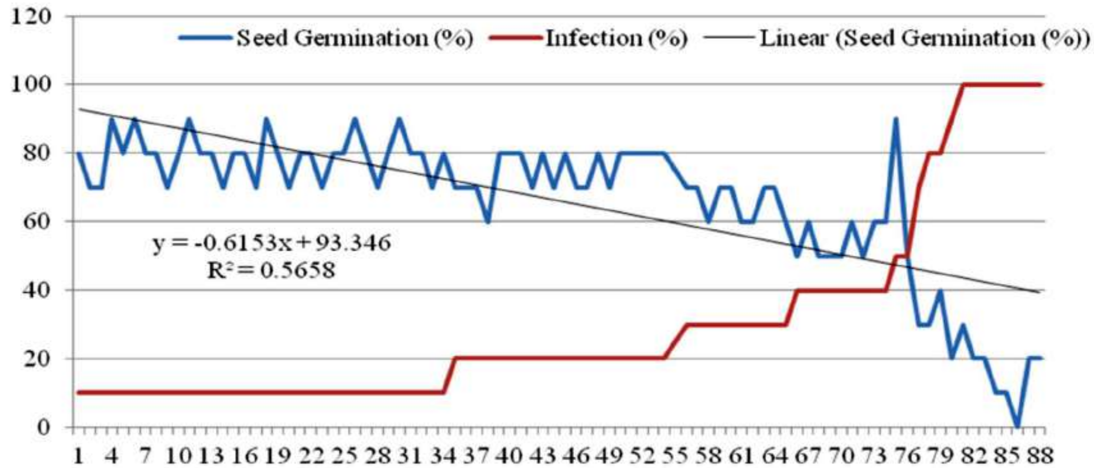

Fig. 4. Linear correlation between seed germination and infection of different fungi detected on medicinal and aromatic plants germplasm

on *Coix lacryma-jobi, Costus speciosus, Ochna lanceolata, Perilla frutescens* and *Tagetus patula* and *T. cucumerina* and *Trichosanthes bracteata*. Therefore, these medicinal and aromatic plants are new host records for the said fungi from India. Thus, our finding highlights the importance of pathogens detected on various MAPs germplasm.

Crop-wise infection revealed that *C. speciosus* is the most vulnerable to seed-borne fungi among MAPs assessed, which was recorded with nine fungal species i.e., *Botrytis cinerea*, *Cephalosporium maydis*, *Fusarium semitectum*, *F. solani*, *F. verticillioides*, *Lasiodiplodia theobromae*, *Melanospora zamiae*, *Myrothecium verrucaria* and *Phoma sorghina* followed by *T. cucumerina* and *T. bracteata* recorded with seven fungal species i.e., *Fusarium oxysporum*, *F. solani*, *F. verticillioides*, *L. theobromae*, *Phoma exigua* var. *exigua*, *P. sorghina* and *Verticillium albo-atrum*.

Accession-wise infection showed that IC-587520 of *C. speciosus* was recorded with highest infection/multiple infections of seven fungal species i.e., *B. cinerea*, *C. maydis*, *F. semitectum*, *F. solani*, *F. verticillioides*, *M. zamiae* and *P. sorghina* followed by IC-439154 with *B. cinerea*, *F. semitectum*, *L. theobromae* and *M. verrucaria* with varying degree of infection level. Location-wise analysis revealed that the maximum numbers of pathogenic fungal species (12) belonging to nine genera were detected on MAPs germplasm from Jharkhand state followed by Uttarakhand state where six fungal species belonging to three important genera on 11 accessions of four crop species of MAPs germplasm. The state of West Bengal was also recorded with five fungal species belonging to four genera from three accessions of two crop species. Therefore, MAPs germplasm may be comparatively more prone to fungal diseases in Jharkhand, Uttarakhand and West Bengal than other states (Table 1).

Out of the total 880 accessions of MAPs germplasm processed for SHT, pathogen-wise overall infection showed share of *B. cinerea* (11.1%), *F. oxysporum* (2.8%), *F. semitectum* (5.6%), *F. solani* (5.6%), *F. verticillioides* (8.3%), *L. theobromae* (8.3%), *M. roridum* (2.8), *P. sorghina* (19.4%), *U. coicis* (44.4%) and *V. albo-atrum* (4.2%) altogether in 71 infected accessions (8.1%) (Fig. 3).

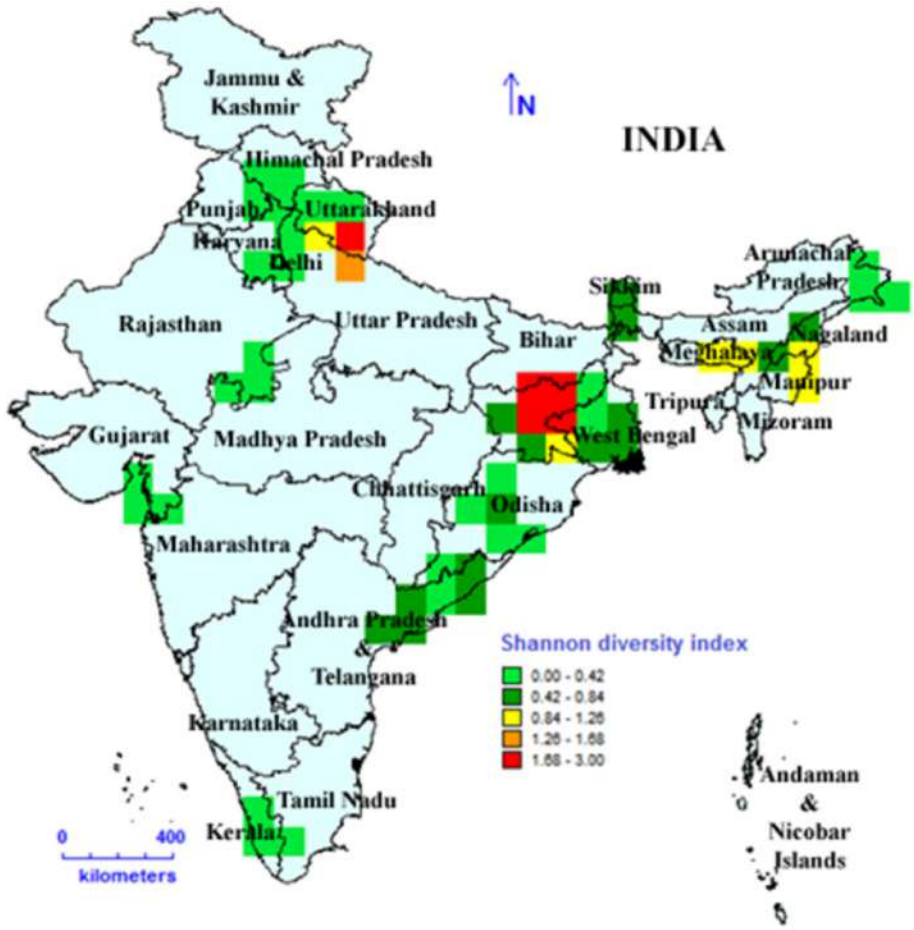

Fig. 5. Shannon's diversity index of fungal pathogens associated with medicinal and aromatic plants germplasm in India

Further, correlation analysis revealed that there is a significant negative correlation between the level of infection and seed germination with r value -0.93 and r^2 value of 0.56 (Fig. 4). GIS based grid map was generated for showing the pathogens distribution in different states of the country. Colours of the grids are indicative of the extent of occurrence of the seed-borne fungal pathogens. Grid map generated revealed the highest pathogen richness in Jharkhand with 12 species belonging to nine fungal genera followed by Uttarakhand with six species belonging to three genera (Fig. 5).

Detection of such a large number of pathogens signifies the role of SHT in the safe conservation of these germplasm. These infected seeds could have been a source of inoculum and spread of the pathogen across the country and could have posed a serious threat to the medicinal and aromatic crops industry if these seeds are conserved and released for utilization in various crop improvement programmes for farmers. Therefore, the seed health testing has significant importance in conserving disease-free material for minimizing the risk of spreading disease in the country.

ACKNOWLEDGEMENTS

The authors are thankful to The Director, ICAR-NBPGR, New Delhi for providing facilities. The authors also acknowledge the help extended by the scientists and technical staff of Plant Quarantine Division in processing the samples for this study. Thanks are also acknowledged to Dr. Dinesh Prasad Semwal, Division of Germplasm Exploration, ICAR-NBPGR for developing pathogens diversity index map.

REFERENCES

Akhtar J, Kandan A, Singh B, Chand D, Kumar J and Agarwal PC (2014). Modified technique of obtaining pure cultures of seed-borne fungi. *Indian J. Pl. Protection* 42: 156-159.

Barata AM, Rochaa F, Lopesa V and Carvalhoba AM (2016). Conservation and sustainable uses of medicinal and aromatic plants genetic resources on the worldwide for human welfare. *Ind. Crops Prod.* 88: 8-11.

CAB International (2007). Crop Protection Compendium (2007th Edition). Centre for Agriculture and Bioscience International. Wallingford, Oxon, UK.

Chandel S, Dubey K and Kaushal P (2014). Major diseases of medicinal and aromatic plants recorded in Himachal Pradesh, India. *J. Pl. Dis. Sci.* 9: 145-153.

Chavan SF and Korekar SL (2011). A survey of medicinal plants for fungal diseases from Osmanabad district of Maharashtra state. *Recent Res. Sci. Technol.* 3: 15-16.

Choudhary S (1946). Some studies on smut *Ustilago coicis* Bref. of Job's tears millet. *J. Ind. Bot. Soc.* 25: 123-130.

Dev U, Agarwal PC, Singh B, Chand D and Bhardwaj J (2010). Fungal pathogens intercepted in imported germplasm of medicinal and aromatic plant and their quarantine significance. In: Souvenir and Abstracts, National Conference on 'Biodiversity of medicinal and aromatic plants: collection, characterization and utilization. Medicinal and aromatic plants association of India, Anand, Gujrat. (TS3-03), pp. 60-61.

Gilbert RL (2003). First report of *Exserohilum pedicellatum* on *Zea mays* in Australia. *Plant Pathol.* 52: 404.

Gupta S, Dubey A and Singh T (2011). *Fusarium semitectum* as a dominant seed-borne pathogen in *Dalbergia sissoo* Roxb., its location in seed and its phytopathological effects. *Indian Journal of Fundamental and Applied Life Sciences* 1: 5-10.

Gupta V, Singh A, Singh BP and Parakh DB (2013). Medicinal plants used in local health care system of Chanawada village, Udaipur, Rajasthan (A case study). *Int. J. Pl. Sci.* 8: 201-204.

Gure A, Slippers B and Stenlid J (2005). Seed-borne *Botryosphaeria* spp. from native *Prunus* and *Podocarpus* trees in Ethiopia, with a description of the anamorph *Diplodia rosulata* sp. *Mycol. Res*. 109: 1005-1014.

Gurib-Fakim A (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Mol. Aspects Med.* 27: 1-93.

Hijmans RJ, Guarino L, Cruz M and Rojas E (2001). Computer tools for spatial analysis of plant genetic resources data: 1. DIVA-GIS. *Pl. Genet. Resour. Newslett.* 127: 15-19.

Hong CF, Tsai SF, Yeh HC and Fan MC (2013). First report of *Myrothecium roridum* causing Myrothecium leaf spot on *Dieffenbachia picta*'Camilla' in Taiwan. *Plant Dis.* 97: 1253.

Ingle A and Rai M (2011). Genetic diversity among Indian phytopathogenic isolates of *Fusarium semitectum* Berkeley and Ravenel. *Adv. Biosci. Biotechnol.* 2: 142-148.

Irwin J, Singh S, Kochman J and Murray G (1999). Pathogen risks associated with bulk maize imports to australia from the United States of America. A report by technical working group 1: Disease risk analysis, for the import of maize from the USA for processing and use as animal feed. 126 p.

Janardhan KK, Ganguly D and Husain A (1964). Fusarium wilt of *Rauvolfia serpentina*. *Curr. Sci.* 33: 313.

Kiecana I, Cegie³ko M and Mielniczuk E (2012). Fungi colonizing the swing material of turfgrasses considering susceptibility of cultivars to selected pathogens. *Acta Sci. Pol. Hortorum Cultus* 5: 153-168.

Magurran AE (1988). Ecological diversity and its measurement, Princeton University Press. Princeton, New Jersey, USA, p. 215.

Mahdizadeh V, Safaie N and Goltapeh EM (2011). Diversity of *Macrophomina phaseolina* based on morphological and genotypic characteristics in Iran. *Plant Pathol. J.* 27: 128-137.

Mathe A (ed.) (2015). Medicinal and aromatic plants of the world. Scientific, Production, Commercial and Utilization Aspects. Springer Technology and Engineering. 460 p.

Mathur SB and Kongsdal O (2003). Common laboratory seed health testing methods for detecting fungi. International Seed Testing Association, Basserdorf, Switzerland. p 425.

Nelson SC (2008). Mango anthracnose (*Colletotrichum gloeosporioides*). *J. Plant Dis.* 48: 1-9.

Nwachukwu EO and Umechuruba CI (1996). Changes in nutritional values of African yam bean seeds due to seed-borne fungi. *Global J. Pure Appl. Sci.* 3: 141-147.

Okunowo WO, Gbenle GO, Osuntoki AA and Adekunle AA (2010). Media studies on *Myrothecium roridum* Tode: A potential biocontrol agent for water hyacinth. *J. Yeast Fungal Res.* 1: 55-61.

Pande S, Galloway G, Gaur PM, Siddique KHM, Tripathi HS, Taylor P, MacLeod MWJ, Basandrai AK, Bakr A, Joshi S, Kishore KG, Isenegger DA, Rao NJ and Sharma M (2006). Botrytis grey mould of chickpea: A review of biology, epidemiology and disease management. *Aust. J. Agric. Res.* 57: 1137-1150.

Prabhu AS and Bedendo IP (1988). Glume blight of rice in Brazil: Etiology, varietal reaction and loss estimates. *Trop. Pest Manage.* 34: 85-88.

Punnithaligam E (1980). Plant diseases attributed to *Botryodiplodia theobromae* Pat. *Bibliotheca* Mycologia, London, v.71, p.1-123.

Raut AS, Kshirsagar DN, Kurade KC, More SW and Ingle ST (2016). Occurrence of major diseases on medicinal and aromatic plants and their relationship with weather parameters. *Bioinfolet*. 13: 409-419.

Richardson MJ (1990). *An Annonated List of Seed-borne Diseases*. Fourth Ed. International Seed Testing Association, Zurich, Switzerland, p. 387.

Samra AS, Sabet KA and Hingorani MK (1963). Late wilt disease of maize caused by *Cephalosporium maydis*. *Phytopathology* 53: 402-406.

Saremi H, Ammarellou A, Marefat A and Okhovvat SM (2008). Binam, a rice cultivar, resistant for root rot disease on rice caused by *Fusarium moniliforme* in Northwest Iran. *Int. J. Bot.* 4: 383-389.

Saremi H, Okhovvat SM and Ashrafi SJ (2011). Fusarium diseases as the main soil borne fungal pathogen on plants and their control management with soil solarization in *Iran. J. Biotech.* 10: 18391-18398.

Schippmann U, Leaman DJ and Cunningham AB (2002). Impact of cultivation and gathering of medicinal plants on biodiversity: Global trends and issues. *In*: Biodiversity and the ecosystem approach in agriculture, forestry and fisheries, Satellite event on the occasion of the Ninth Regular Session of the Commission on Genetic Resources for Food and Agriculture. Rome, 12-13 October 2002. Inter-Departmental Working Group on Biological Diversity for Food and Agriculture, Rome. pp 1-21. (http://www.fao.org/3/contents/11fc3667-52a7-5192-8fbb-358daaeaf 558/AA010E00.pdf)

Sharma DDK, Bharti YP, Singh PK, Shukla DN and Kumar A (2014). Studies on prevalence and identification of new races of *Fusarium moniliforme* Sheldon incitant of Pokkah boeng disease from Uttar Pradesh. *Global Journal of Biology, Agriculture and Health Sciences* 3: 53-61.

Sharma M and Kulshrestha S (2015). *Colletotrichum gloeosporioides*: an anthracnose causing pathogen of fruits and vegetables. *Biosci. Biotechnol. Rese. Asia* 12: 1233-1246.

Singh B, Akhtar J, Chand D, Sharma R, Kapoor ML and Agarwal PC (2014). Interception of *Phoma sorghina* on Transgenic *Arabidopsis thaliana* from USA- A New Host Record. *Indian J. Plant Protec.* 42: 89-90.

Sinha P, Govil JN and Singh NK (2002). Recent progress in medicinal plants: Diseases and their management. Science & Technology Publishing LLC, USA. 212 p.

Titatarn S, Chiegkul A, Unchalisangkas D, Chamkrachang W, Chew-Chin N and Chandrasrikul A (1983). Occurrence of *Ustilago coicis* on *Coix lacryma-jobi* in Thailand. *Plant Dis.* 67: 434-435.

Yang Shaw-Ming (1995). Host range determination of *Myrothecium verrucaria* isolated from leafy spurge. *Plant Dis.* 79: 995-997.