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Interception of pathogens during quarantine processing: an effort towards safe import of oilseed and vegetable *Brassicas* germplasm in India

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Abstract

During 1976-2015, a total of ~75000 seed samples of oilseed and vegetable Brassicas germplasm received from different countries were processed for quarantine clearance. Seed health testing resulted in interception of 17 pathogenic fungi and one bacterium in 2628 samples from 23 countries. Decade wise (1976-1985; 1986-1995; 1996-2005; 2006-2015) analysis revealed the highest level infections in 948 (36.3%) during 1986-1995 followed by 912 interceptions (34.9%) during 1996-2005 and lowest level of infections was intercepted in 176 samples (6.7%) during 2005-2016. Risk analysis of the interceptions showed that among the pathogens, *Alternaria brassicicola* was recorded in most of the infected samples (65.5%) followed by *Xanthomonas campestris* pv. *campestris* (17.8%). Among countries, maximum interceptions were made from USA (26.3%) followed by Canada (24.0%) which indicated that there is the highest risk of introduction of pathogens along with Brassicas seeds from USA and Canada. Among pathogens intercepted, *Leptosphaeria maculans* causing black leg from Australia and Canada and *X. c.* pv. *campestris* causing black rot of crucifers from Canada are potential quarantine pathogens to India, hence the samples infected with *L. maculans* and *Fusarium solani* were rejected and incinerated and samples infected with *X. c.* pv. *campestris* were salvaged by giving hot water treatment at 50^0C for 20 min. before their release. Whereas, samples infected with other pathogens viz., *A. brassicae*, *A. brassicicola*, *A. raphani*, *A. solani*, *Bipolaris sorghicola*, *B. sorokiniana*, *Botrytis cinerea*, *Cephalosporium maydis*, *F. oxysporum*, *F. verticillioides*, *Phoma sorghina*, *Rhizoctonia bataticola*, *Sclerotium rolfsi*, *Verticillium albo-atrum* were salvaged using fungicidal seed treatment before their release. This could finally prevent entry or minimized spread of exotic pathogens into the country and also promoted germplasm exchange for crop improvement. The interceptions of pathogens of quarantine significance to India from different countries emphasizes the need of critical examination of imported oilseed and vegetable Brassicas during the quarantine processing to safeguard our experimental as well as agricultural fields from inadvertent introduction of associated pathogens or more virulent races/ strains of the existing ones in the country.

Keywords: *Brassicas, germplasm, seed health test, seed-borne pathogen, quarantine*

Introduction

Global exchange of germplasm has a significant role in crop improvement programmes and in boosting our agricultural production as it provided a wide genetic diversity. To prevent inadvertent introduction of pathogens of quarantine significance including the ones not known to occur in India or having economic significance or physiological races or wide host range harmful to the agriculture of our country, ICAR-NBPGR, New Delhi, is the nodal agency for quarantine processing of introduced germplasm for research purpose (Singh *et al.*, 2006, Dev *et al*., 2012).

Brassicaceous oilseeds and vegetables are economically important crops, which get severely affected by several diseases/ pathogens namely, Alternaria blight caused by various species of *Alternaria*, namely, *A. barssicae*, *A. brassicicola*, *A. solani*, *A. raphani*, black leg (*Leptosphaeria maculans*) and black rot of crucifers (*X. c.* pv.

campestris) in different geographical regions of the world (CABI, 2007). These pathogens may result in poor quality seed, loss in germination, development of plant disease and spread of new strains or physiological races of the pathogen(s) along with seeds to new geographical areas through transboundary movement of infected seeds. While processing the material for quarantine clearance through seed-health testing in the past, a number of pathogens including the ones having economic significance or physiological races or wide host range have been intercepted (Singh *et al.,* 2007, Dev *et al*., 2012). Therefore, plant quarantine as biosecurity tool assumes special importance in order to protect our experimental farms as well as agricultural fields from inadvertent introduction of pathogens or more virulent races/ strains of the existing ones in the country associated with Brassicas germplasm and risk assessment of the pathogens intercepted in imported Brassicas germplasm during past four decades is discussed in this paper.

Materials and Methods

Seed health testing of seed samples (~75000) of Brassicas germplasm introduced from 23 countries were carried out at the Division of Plant Quarantine, ICAR-NBPGR, New Delhi, India during 1976 to 2015. During seed-health testing, all the seed samples were first examined visually. Later, seeds were subjected to incubation test using blotter technique. The seeds were placed on 3 layers of moist blotters in 110 mm labeled plastic Petri plates (seeds/plate varied from 10 to 25, depending on quantity in of the sample) and incubated for 7 days at 22±1^{0}C under alternating cycles of 12 hr light and darkness. Observations for presence of seed-borne pathogens were recorded on the 8th day under stereo-binocular microscope at different levels of magnification i.e. 0.75X to 11.25X. Pathogens those sporulated on seeds were particularly identified as per the characteristics described in IMI descriptions of fungi by Mathur and Kongsdal (2003) and colony characters including conidial arrangement and slides were also prepared and confirmed under compound microscope at different levels of magnification i.e. 4.0 X to 40.0 X, whenever required. Further observations were made on morphological characteristics such as formation of conidia and conidiophores under stereo-binocular microscope and shape and size of conidia under compound microscopes and their frequency of occurrence. For detection of bacterium, *Xanthomonas c.* pv. *campestris* in Brassicas, seedlings showing 'V' shaped lesions in the blotter test were cut with a sharp sterilized blade from the infected part of the cotyledon/ seedling, mounted in water drop and examined under compound microscope. A slow to fast oozing of bacteria from vascular bundles indicated the bacterial association with infected portion (Singh *et al*., 2006). The bacterium was isolated on nutrient-agar medium and examined after 72 hours of incubation for identification. Finally data was tabulated to analyze the risk of pathogens associated with them while introducing crop germplasm during 1976 – 2015.

Results and Discussion

Critical laboratory examinations of seed samples of imported Brassicas germplasm using blotter test could ensure the identification of 17 seed-borne fungi one bacterium. Based on their morphological characteristics/ growth observed on seeds/ seedlings as described by IMI descriptions for fungi and bacteria, Mathur and Kongsdal (2003) and Shekhawat *et al*. (1982), they were identified as *A. brassicae* (Berk.) Sacc., *A. brassicicola* (Schwein.) Wiltshire, *A. raphani* Groves and Skolko, *A. solani* Sorauer, *Botrytis cinerea* Pers.: Fr. (Tel: *Botryotinia fuckeliana* (de Bary) Whetzel), *B. sorghicola* (Lefebvre & Sherwin) Alcorn, *B. sorokiniana* (Sacc.) Subram. & Jain, *Cephalosporium maydis* Samra, Sabet & Hingorani, *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen, *F. solani* (Martius) Sacc., *F. verticillioides* (Sacc.) Nirenberg, *Leptosphaeria maculans* (Desm.) Ces. & De Not. (syn: *Phoma lingam* (Tode ex Fr) Desm), *Phoma sorghina* (Sacc.) Boerema, Dorenb. & Kesteren, *Rhizoctonia bataticola* (Taub.) Butler, *Sclerotium rolfsi* Saccardo, *Verticillium albo-atrum* Reinke & Berthold and *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson in 2629 samples from 23 countries (Table 1).

Among pathogens, overall interceptions revealed that infection of *Alternaria brassiciola* was the highest

Table 1: Morphological features of fungi and bacterium intercepted in Brassicas germplasm introduced during 1976-2015

Shape/ type	Growth characteristics		Attachment	Genus
	Septation	Dimension		
Conidia mostly straight, obclavate and rostrate	6-9 transverse and 0-8 longitudinal septa	Length 75-350 and width 20-30 µm	Solitary conidial arrangement	A. brassicae
Cylindrical usually tapering slight towards the apex, basal cell rounded, pale to dark olivaceous brown	Mostly transverse septa (1-11)	Length 18-130 and width 8-10 µm	Conidia arranged mostly in chains	A. brassicicola
Conidia mostly obclavate or ellipsoidal with short beak	2-7 transverse and usually some longitudinal and oblique septa	Length 50-130 and width 14-30 µm	Conidia mostly attached to conidiophores singly	A. raphani
Conidia obclavate, oblong or ellipsoidal tapering in long beak, usually of the same length or sometime longer than conidia	9-11 transverse septa and base of the conidia conical and narrow	Length 150-300 and width 15-19 µm	2-3 conidia arranged solitary on each conidiophore	A. solani
Conidia ellipsoid, mostly straight, sometimes slightly curved, tapering towards rounded ends	6-8 distoseptate	Length 50-85 µm and width 12-15 µm	Conidia arranged on conidiophores acro-pleurogenous manner	*B. sorghicola* (Fig. 1d-e)
Conidia ellipsoid, mostly straight, thick-walled with rounded ends	Mostly 9-10 distoseptate	Length 60-90 µm and width 18-20 µm (at the broadest part)	Conidiophores erect, short and single bearing 1-6 conidia arranged in acrople-urogenous manner	B. sorokiniana
Conidiophores with characteristic 'twisting' at several places	Single-celled with slight protuberant hilum	Length 8-14 µm and width 6-9 µm	Bearing ashgrey to greyish clusters of conidia at several places	*B. cinerea* (Fig. 1f-g)
Conidia hyaline, ellipsoidal to cylindrical, straight with rounded ends	Conidia single-celled	Length 3-10 µm and width 1.5-3 µm	Conidia in the form of shiny, round and watery heads attached perpendicular to conidiophores	C. maydis
Microconidia oval elliptical and macroconidia falcate along with chlamydospores	Microconidia mostly non septate and macroconidia mostly 3-septate	Microconidia 5-8 µm long and 2-3 µm wide; macroconidia mostly 45-50 µm long and 3 µm wide	Microconidia formed in false-heads on monophialides macro-conidia in slimy mass	*F. oxysporum*
Microconidia hyaline, oval, ellipsoidal or reniform and macroconidia thick-walled, hyaline with short rounded apical cell.	Microconidia mostly 0-1 septate and macroconidia mostly 3-4 septate	Microconidia 5-8 µm long and 2-3 µm wide; macroconidia mostly 45-50 µm long and 3 µm wide	Microconidia formed in translucent to opaque, milky white watery drops on long phialides; macro-conidia produced in sporodochia	F. solani

Pycnidia brown to dark brown	Non-septate, hyaline, sometimes guttulate	Conidia ranged from 3.5-4.5 x 1.5 µm	Pycnidia erumpent with silvery white mycelium	*L. maculans* (Fig. 1a-c)
Profuse mycelium, pycnidia black and shiny with small to long neck	Non-septate, hyaline, globose and gutulate	Conidial dimension ranged from 1.5-2.5 x 4.5x6.5 µm	Pycnidia superficial or on aerial mycelium	P. sorghina
Only radiating thick mycelium without sclerotia	Formation of septum in the mycelial branch near the point of origin with dolipore septum, constriction of the branch at the origin point.	-	Branching from parent hypha is at right angle and when cultured on medium, sclerotia not differentiated into rind and medulla	R. bataticola
Spherical, mall, black microsclerotia	Septa formed in the mycelium near the point of origin without clamp connection	Sclerotia mostly 1-2 mm across	Small, black micro-sclerotia	S. rolfsii
Verticils of phialieds bearing small colourless, circular, shiny watery drops at the tips of each vertical.	Single-celled, occasionally 1-septate	Conidial length 3.5-10.5 µm and width 2.0-4.0 µm	Verticils of phialides arranged erect on conidiophores	V. albo-atrum
The bacterial colonies were yellow, raised, convex, shiny and mucoid on yeast glucose chalk agar medium	Gram-negative, rod-shaped, did not reduce nitrates but hydrolyzed starch, casein and gelatin	0.7-3.0 × 0.4-0.5 ìm, motile with a single polar flagellum	The bacterium produced acid from arabinose, dextrose, alactose, glycerol, maltose, mannitol, raffinose and sac-charose	*X. c.* pv. *campestris*

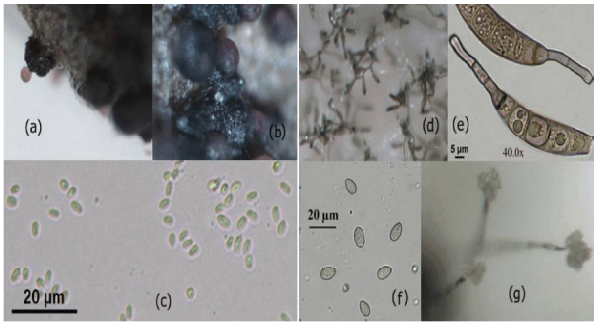

Fig.1: Growth characteristics of *Leptosphaeria maculans* (a-c), *Bipolaris sorghicola* (d-e) and *Botrytis cinerea* (f-g) observed on Brassicas seeds imported from different countries.

in 1723 samples (65.5%) from 21 countries followed by *X. c.* pv. *campestris* in 469 samples (17.8%) from 12 countries, *Alternaria brassicae* in 194 samples (7.4%), *F. solani* and *L. maculans* in 74 samples each (2.8% each), *V. albo-atrum* in 17 samples (0.6%) and rest of the pathogens were intercepted in 77 samples (2.9%) (Table 2, Fig. 2).

A. brassicae, causing Altenaria blight/ leaf blight of crucifers, was intercepted in number of Brassicas from nine countries, namely Canada, Ethiopia, Finland, Italy, Netherlands, Sweden, Taiwan, UK and USA countries with the highest infection from Canada in 75 samples (38.7%) followed by Sweden in 69 samples (35.6%) (Table 2).

A. brassicicola, causing Alternaria blight/ black spot of crucifers/ brown rot of cabbage, was intercepted from 21 countries viz., Australia, Belgium, Canada, China, Denmark, Ethiopia, France, Germany, Hungary, Italy, Japan, Korea, Nepal, Netherlands, New Zealand, Philippines, Russia, Sweden, Taiwan, UK and USA with the highest interception from Canada in 442 samples (25.7%) followed by USA in 425 samples (24.7%) (Table 2). Prasad and

Table 2: Country-wise interceptions of different pathogens in oilseed and vegetable Brassicas germplasm imported during 1976-2015

Country	Interception (No.) of different pathogens							
	Ab(194)**	*Abc*(1723)	*Fs*(74)	*Lm*(74)	*Va*(17)	*Xcc*(469)	Others(77)	Total(2629)
Australia	12(6.2)***	134(7.8)	0(0.0)	25(33.8)	0(0.0)	34(7.2)	6(7.7)	211
Canada	75(38.7)	442(25.7)	4(5.4)	31(41.9)	0(0.0)	49(10.4)	30(38.5)	631
Italy	8(4.1)	38(2.2)	0(0.0)	12(16.2)	0(0.0)	44(9.4)	0(0.0)	102
Netherlands	8(4.1)	107(6.2)	1(1.4)	1(1.4)	15(88.2)	30(6.4)	19(24.4)	181
Russia	0(0.0)	85(4.9)	2(2.7)	1(1.4)	0(0.0)	0(0.0)	2(2.6)	90
Sweden	69(35.6)	98(5.7)	3(4.1)	1(1.4)	0(0.0)	28(6.0)	2(2.6)	201
Taiwan	6(3.1)	159(9.2)	14(18.9)	0(0.0)	0(0.0)	9(1.9)	1(1.3)	189
UK	5(2.6)	124(7.2)	8(10.8)	3(4.1)	0(0.0)	34(7.2)	2(2.6)	176
USA	3(1.5)	425(24.7)	29(39.2)	0(0.0)	1(5.9)	228(48.6)	4(5.1)	690
Others*	8(4.1)	111(6.4)	13(17.6)	0(0.0)	1(5.9)	13(2.8)	12(15.4)	158

Ab = A. brassicae; Abc = A. brassicicola, Fs = F. solani; *Lm = L. maculans*; *Va = V. albo-atrum*; *Xcc = X. c.* pv. *Campestris*
*Others include *A. raphani, A. solani, B. sorghicola, B. sorokiniana, B. cinerea, C. maydis, F. oxysporum, F. verticillioides, Phoma sorghina, R. Bataticola* and *S. rolfsi*
**Values in parenthesis are total number of infected samples intercepted from importing countries
***Values in parentheses are interceptions (%) of different pathogens among countries

Vishunavat (2006) reported maximum loss (50.0%) in seed test weight in cauliflower seed crop due to Alternaria blight (*A. brassicae* and/ or *A. brassicicola*). Kumar (1997) reported yield loss of 27.5% in *B. rapa* var. *yellow sarson*, 25.0% in *B. rapa* var. *brown sarson* and 20.3% in *B. juncea* due to Alternaria blight from Himachal Pradesh, India. Shrestha *et al.* (2005) reported average yield losses in the range of 32-57 per cent due to Alternaria blight in mustard from Nepal. *A. brassicicola* has been responsible for yield loss up-to 50% in rape in Germany (MacKinnon *et al.*, 1999). Hossain and Mian (2005) reported seed yield loss of 59% in cabbage due to Alternara blight (*A. brassicicola*) in Bangladesh. Black spot, caused by these two fungi, is a major disease in the Netherlands and other European countries, which causes yield losses up-to 75% in different crops (CAB International, 2007).

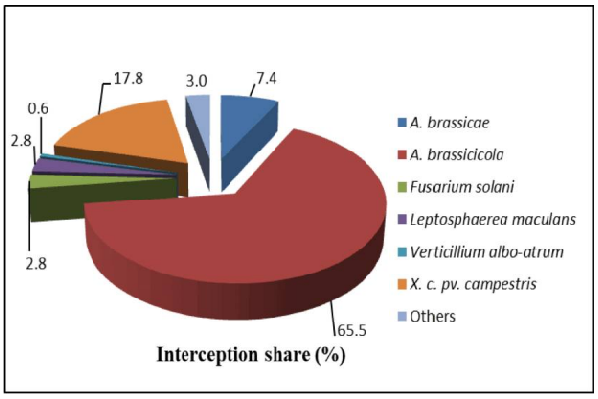

Fig. 2: Pathogen-wise share of interceptions in oilseed and vegetable Brassicas germplasm imported during 1976-2015

A. raphani, causing leaf spot in crucifers, was intercepted in one sample of *B. rapa* from Australia, in two samples of *B. carinata* from Germany and in 16 samples of *Brassica* spp. from Canada. Vannacci and Pecchia (1988) detected *A. raphani* on 80% in a seed lot of *Raphanus sativus* and a large proportion of diseased seedlings died before emergence due to this fungus. In a Canadian study, Rude *et al.*, 1999 intercepted *A. raphani* in seed samples of *Brassica rapa* from Saskatchewan and Alberta which sigificantly reduced seed germination.

A. solani Sorauer, causing early blight, is one of the most common and serious diseases of potato and tomato, which causes economic losses, was intercepted in two samples of *B. napus* imported from Canada. The hosts of *A. solani* include *Solanum lycopersicum S. tuberosum, S. melongena. S. carolinensis, S. nigrum, Capsicum frutescens, B. oleracea, Cucumis sativus* and *Zinnia elegans* (Pscheidt 1985).

B. sorghicola was intercepted in one sample of *B.*

o. var. *botrytis* from Taiwan. It has been reported to be seed-borne only on maize, sorghum and Sudan grass causing Target leaf spot (CAB International 2007). Its interception in only one sample of *B. o.* var. *botrytis* constitutes new host record.

B. sorokiniana, the most important foliar pathogen of wheat and barley, was intercepted in two samples each of *B. juncea* and *B. napus* from Australia and in one sample each of *B. juncea* from Canada and *B. napus* from USA. The disease is a major biotic constraint in most of the wheat growing areas. There are many physiological races and geographically remote populations reported to differ in virulence. Losses due to its infection in the barley production during 2006 to 2009 were estimated to range from 30% to 70% of yield in Macedonia (Karov *et al.,* 2009).

B. cinerea, a haploid necrotrophic fungal pathogen, was intercepted in one sample each of *B. juncea* from Sweden and *B. napus* from Germany, *B. rapa* from USA, *B. oleracea* var. *botrytis* from Netherlands and *Brassica* spp. from Sweden and UK. This causes botrytis rot, grey mould diseases in over 200 plant species of economic importance including chickpea (Pande *et al.,* 2006), Weiberg *et al*. (2013) also reported that *B. cinerea* infects almost all vegetable and fruit crops and caused annual losses of 10 to 100 billion US dollars worldwide.

C. maydis, causing black bundle disease / late wilt of maize, was intercepted in three samples of *B. juncea* from Canada, 10 samples of *B. o.* var. *botrytis* from Netherlands and in one sample from UK and in one sample of *B. o.* var. *italica* from Netherlands, which is internally and externally seed-borne pathogen on limited host comprising maize, cotton and lupins with limited geographical distribution including India, Egypt and Hungary (CAB International 2007). Its widespread incidence and severity in Egypt, with 100% infection has been reported in some fields. It was considered potentially an important pathogen (http:/ cropgenebank.sgrp. cgiar.org/index.php?option= com_content&view= article &id=443&Itemid=625). Its interception in *Brassica* spp. revealed a new host record.

F. oxysporum, a causal agent of vascular wilt in many crops, was intercepted in one sample of *B. juncea* from Canada. The pathogen is of great economic importance as it causes substantial crop losses in most of the host crops worldwide. High genetic diversity has been reported in this fungus throughout the world (Kim *et al*., 2005, Abo *et al*., 2005). Therefore, there is high risk of introduction of a new or more virulent race in the country, which may cause severe losses to the crops on which intercepted.

F. solani, causing wilt and damping-off on a number of crop species, was intercepted from 11 countries, namely Canada, China, Denmark, France, Hungary, Netherlands, Russia, Sweden, Taiwan, UK and USA with the highest interception from USA in 29 samples from USA (39.2%) followed by Taiwan in 14 samples (18.9%) of oilseed and vegetable Brassicas (Table 2). Saremi *et al*. (2011) reported yield losses to the extent of 30.0 to 70.0% in the fields due to *F. solani*. It causes substantial economic losses world over and molecular studies revealed high level of diversity within the fungus (CAB International 2007).

F. verticillioides, causing bakane/ stalk/ stem/ ear rot diseases, was intercepted from five countries. The infection of *F. verticillioides* was intercepted in one sample each of *B. juncea* from Canada and *B. napus* from Australia, in *B. o.*var. *botrytis* from Korea (eight samples) and Netherlands (four samples) and in *Brassica* spp. from USA (one sample). Hossain *et al*. (2013) also reported 51.53 and 37.60% yield reduction in Aus and Aman rice, respectively from Bangladesh. It has been reported to be seed-borne on several hosts (Singh *et al*. 2015). Vigier *et al.* (2001) reported crop losses of 48.0% in maize while in wheat, the losses were up to 70.0%. Saremi *et al.* (2008) reported yield losses up to 75.0% in rice from Iran. This fungus is reported to have wide genetic variability (Gohari *et al*. 2008; Sharma *et al.,* 2014).

L. maculans, a fungus causing black leg in crucifers, was intercepted from only six countries, namely Australia, Canada, Italy, Netherlands, Russia, Sweden and UK with the highest interception in 31 samples from Canada (41.9%) followed by Australia

(33.8%) in 25 samples (Table 2, Fig. 2). It was intercepted in 27 samples of *B. juncea* from Canada and in 24 samples from Australia, in one sample each of *B. napus* from Australia, Canada and UK, in one sample of *B. o.* var. *botrytis* from Netherlands and in two samples each of *B. o.* var. *botrytis* from UK, and *B. rapa* from Canada and in one sample each of *Brassica* spp. from Italy, Russia and Sweden, in two samples from Canada and in 11 samples from Italy. Hammoudi *et al.* (2012) reported yield losses up to 95% due to black leg in oilseed rape.

P. sorghina, causing leaf spot of sorghum/ glume blight in rice, was intercepted in one sample of *B. o.* var. *botrytis* from Korea. This fungus has wide host range and worldwide distribution. Prabhu and Bedendo (1988) reported yield losses upto 14.0 per cent due to glume blight caused by *P. sorghina* in rice. In Brazil, glume blight was considered to be of minor economic importance earlier, but attained epidemic proportions in rice over a large geographical area in 1979–80. Its interception in *Brassica* spp. revealed a new host record.

R. bataticola, causing dry root rot of chickpea and wilt in linseed, was intercepted in two samples of *B. napus* from USA and one sample of *Brassica* spp. from Russia. Dry root rot in chickpea and wilting in linseed due to *R. bataticola* are one of the serious problems and has been found associated with seeds of chickpea and linseed, respectively. Its interception in *B. napus* from USA revealed a new host record.

S. rolfsii, causing damping-off/ collar rot/ root rot/ stem rot/ fruit rot/ leaf spot/ neck rot, was intercepted in one sample of *B. o.* var. *botrytis* from Netherlands. It infects more than 500 plant species, but is especially severe on legumes, solanaceous crops, cucurbits and other vegetables grown in rotation with beans (Hall, 1991). There is no evidence for seed transmission of the pathogen. But, it has been detected in soybean seeds at infection levels of up to 42% (Popoola and Akueshi, 1986). groundnu. Other seeds on which the pathogen has been detected include *Lens culinaris*, *Phaseolus vulgaris* (Akem and Dashiell, 1991), *Triticum aestivum*, lettuce, jute, periwinkle and watermelon, etc. (Ikediugwu, 1980). Its interception in *B. o.* var.

Table 3: Country-wise interceptions among pathogens in oilseed and vegetable Brassicas germplasm imported during 1976-2015

Pathogen	Interception of pathogens (No.) from source of import								
	Australia (211)*	Canada (631)	Italy (102)	Netherlands (181)	Russia (90)	Sweden (201)	Taiwan (189)	UK (176)	USA (690)	Others (158)	Total(2628)
A. brassicae	12(5.7)**	75(11.9)	8(7.8)	8(4.4)	0(0.0)	69(34.3)	6(3.2)	5(2.8)	3(0.4)	8(5.1)	194
A. brassicicola	134(63.5)	442(70.0)	38(37.3)	107(59.1)	85(94.4)	98(48.8)	159(84.1)	124(70.5)	425(61.6)	111(70.3)	1723
F. solani	0(0.0)	4(0.6)	0(0.0)	1(0.6)	2(2.2)	3(1.5)	14(7.4)	8(4.5)	29(4.2)	13(8.2)	74
L. maculans	25(11.8)	31(4.9)	12(11.8)	1(0.6)	1(1.1)	1(0.5)	0(0.0)	3(1.7)	0(0.0)	0(0.0)	74
V. albo-atrum	0(0.0)	0(0.0)	0(0.0)	15(8.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.1)	1(0.6)	17
X. c. pv. *campestris*	34(16.1)	49(7.8)	44(43.1)	30(16.6)	0(0.0)	28(13.9)	9(4.8)	34(19.3)	228(33.0)	13(8.2)	469
Other	6(2.8)	30(4.8)	0(0.0)	18(10.0)	2(2.2)	2(1.1)	1(0.5)	2(1.1)	4(0.6)	12(7.6)	77

*Values in parenthesis are number of infected samples intercepted from importing countries

**Values in parentheses are interceptions (%) among pathogens from different countries

botrytis from Netherlands revealed a new host record.

V. albo-atrum, a fungus causing wilt in various economically important crop species, was intercepted from Germany, Netherlands and USA with the highest interception in 15 samples *B. o.* var. *botrytis* from Netherlands (88.2%) (Table 2, Fig. 2). Gent *et al*. (2012) reported the outbreaks of *V. albo-atrum* on hop in Oregon with 29.3 and 19.7 per cent wilt incidence during 2006 and 2007, respectively, which were not known to occur in Oregon. The fungus is known to possess a number of physiological strains (CAB International, 2007).

X. c. pv. *campestris*, the causal agent of black rot of crucifers, was intercepted from 12 countries viz., Australia, France, Germany, Hungary, Italy, Nepal, Netherlands, Russia, Sweden, Taiwan, UK and USA with the highest interception in 228 samples from USA (48.6%) followed by Italy in 44 samples (9.4%). The bacterium is reported to survive in seeds up to three years (CAB International, 2007) and seed infection as low as 0.03% can cause epidemic in a field (Vicente *et al.*, 2001). Fargier and Manceau (2007) reported existence of nine races in *X. c* pv. *campestris*. In past, *X. c.* pv. *campestris* has been intercepted in oilseed and vegetable Brassicas from 38 countries (Singh *et al.*, 2006).

Country-wise analysis revealed the highest number of infected samples (690) intercepted from USA (26.3%) followed by Canada with 631 samples (24.0%), Australia with 211 samples (8.0%), Sweden with 201 samples (7.6%), Taiwan with 189 samples (7.2%), Netherlands with 181 samples (6.9%), UK with 176 samples (6.7%) and remaining 231 samples intercepted from other countries (Fig. 3).

The interceptions from Australia revealed that out of the total 211 samples infected, infection of *A. brassicicola* was intercepted highest in 134 samples (63.5%) followed by *L. maculans* in 25 samples (33.8%). The interceptions from Canada revealed that out of the total 631 samples infected, infection of *A. brassicicola* was again highest in 442 samples (63.5%) followed by *X. c.* pv. *campestris* in 49 samples (33.8%) Similarly, interceptions from USA revealed that infection of *A. brassicicola* was again highest in 425 samples (61.6%) followed by *X. c.* pv. *campestris* in 228 samples (33.0%).

Decade wise (1976-1985; 1986-1995; 1996-2005; 2006-2015) analysis revealed the highest infections of various pathogens were intercepted during 1986-1995 in 948 samples (36.3%) followed by 912 samples (34.9%) during 1996-2005, 593 samples (22.7%) during 1976-1985 and the lowest infections was intercepted during 2005-2016 in 176 samples (6.7%) (Fig. 4).

Pathogen-wise analysis showed the highest infection of *A. brassicae* and *A. brassicicola* with interception in 84 (43.3%) and 757 (43.9%) samples, respectively during 1996-2005. Whereas, the highest infections of *Fusarium solani* and *X. c.* pv. *campestris* were intercepted during 1986-95 in 42 (56.8%) and 200 (42.6%) samples, respectively. The highest infection of *L. maculans* was intercepted in

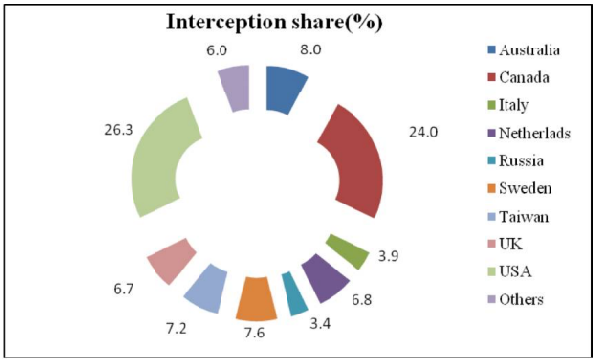

Fig. 3: Country's share in overall interceptions of pathogens in oilseed and vegetable Brassicas during 1976-2015.

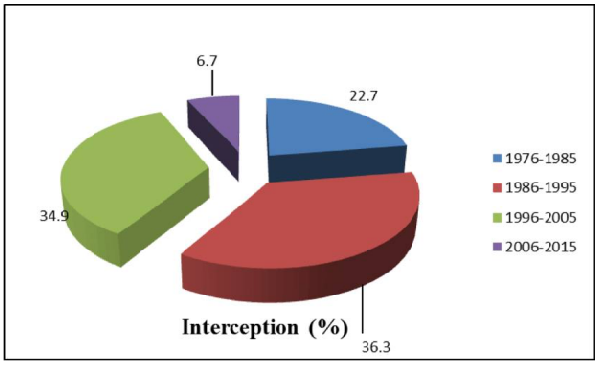

Fig. 4: Decade wise overall interceptions of the pathogens in oilseed and vegetable Brassicas germplasm imported during 1976-2015

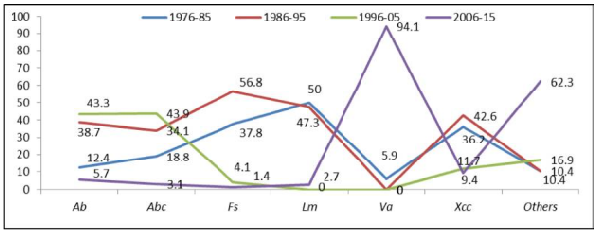

Fig. 5: Decade-wise interception (%) of pathogens during 1976-2015

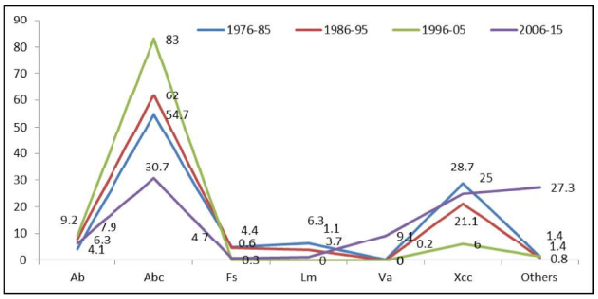

Fig. 6: Pathogen-wise interception during different decades

37 samples (50.0%) of almost all the species of oilseed and vegetable *Brassica* during 1976-85 (Table 4, Fig. 5).

Decade-wise interceptions revealed that during 1976-85, the highest infection (54.7%) was intercepted with *A. brassicicola* followed by *X. c.* pv. *campestris* (28.7%). During 1986-95, the highest infection was again intercepted with *A. brassicicola* (62.0%) followed by *X. c.* pv. *campestris* (21.1%). Similarly, during 1996-2005 most of the infections were intercepted with *A. brassicicola* (83.0%) and during 2006-15, infections of mainly *A. brassicicola* (30.7%) and *X. c.* pv. *campestris* (25.0%) were intercepted (Fig. 6).

Further analysis of overall interceptions revealed that *A. brassicicola* was the most consistently intercepted fungus in 1723 samples of oilseed and vegetable Brassicas imported from 23 countries followed by *X. c.* var. *campestris* in 469 samples of from 9 countries.

Critical laboratory examinations could ensure the interception and identification of associated pathogens in imported oilseed and vegetable Brassicas germplasm which later facilitated in selecting the appropriate salvaging methods for target pathogens to make the infected germplasm free from infection. Infected samples were salvaged prior to release. Introduction of such infected germplasm into the country, otherwise, would have caused additional threat to concern crops. This could finally prevent entry of pathogens of quarantine significance to India. Therefore, large number of interceptions of oilseed and vegetable Brassicas germplasm introduced from 23 countries highlights the magnitude of seed-borne aspect and highlighted the need of quarantine processing critically as phyto-sanitary tool through seed health testing to safeguard our experimental as well as agricultural fields from inadvertent introduction of associated pathogens or more virulent races/strains of the existing ones in the country.

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Table 4: Decade-wise pathogens infection intercepted during 1976-2015

Pathogen	Interceptions (No.)				
	1976-85	1986-95	1996-05	2006-15	Total
A. brassicae	24	75	84	11	194
A. brassicicola	324	588	757	54	1723
F. solani	28	42	3	1	74
L. maculans	37	35	0	2	74
V. albo-atrum	1	0	0	16	17
X. c. pv. *campestris*	170	200	55	44	469
Others	8	8	13	48	77
Total	592	948	912	176	2628

contribution of retired Scientists/ Technical Officers of Plant Pathology Section and staff of Plant Quarantine Division in processing the samples for quarantine clearance.

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