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Occurrence and Distribution of Major Seedborne Fungi Associated with *Phaseolus* Bean Seeds in Ethiopia

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ABSTRACT

A total of 245 seed samples of *Phaseolus* bean; 172 common beans, 51 climbing beans and 22 green beans were collected from various bean growing areas during 2003 crop season. The incidence and severity of seed infection by the major fungal diseases of bean varied between localities, bean types and cropping practices. Thirteen seed-borne fungal pathogens of different genus were identified from seed samples collected from the major bean growing regions of Ethiopia. The incidence of different seedborne fungi ranging between 0.2 to 14.5% was found to vary from location to location and growing conditions. Among them, *Colletotrichum lindemuthianum*, *Phaeoisariopsis griseola,* and *Ascochyta phaseolorum* were the most widespread and damaging seedborne fungal pathogens associated with *Phaseolus* bean seeds in Ethiopia. From the total seed samples collected, 26.2%, 19.6% and 13.6% of common bean, climbing bean and green bean respectively were infected by *C. lindemuthianum*, whereas infection by *P. griseola* was 18.6% and 15.7% on common bean and climbing beans seeds respectively. Green bean seeds were not infected by the latter two fungi. Seeds collected from south, southwest, and western part of Ethiopia showed heavy seed infection by these major fungal pathogens, whereas seeds produced in dry areas with minimum rainfall or under irrigation showed very low seed infection. *Phytophthora* rot of beans caused by *Phytophthora* sp. was also detected from green bean pods and immature seeds produced under irrigation in the central rift valley of the country. The geographic distribution of major seed-borne fungi of *Phaseolus* beans was mapped.

Key words: *Phaseolus* beans, seedborne fungi, distribution, Ethiopia

INTRODUCTION

Different types of *Phaseolus* beans are widely cultivated in various agro-ecological regions of Ethiopia. The most important *Phaseolus* beans grown in the country are dry common beans (*Phaseolus vulgaris* L), climbing beans (*Phaseolus coccineus*) and French/green beans (*Phaseolus vulgaris*). The area covered by dry common bean production alone in Ethiopia is estimated to be more than 200,000 hectare annually (CSA, 2000).

Among the biotic stresses of *Phaseolus* bean production in the country, diseases are considered the major production threats. Under optimum crop production management practices of Ethiopia, there is a potential to produce common beans of 2.5 - 3.0 tons/hectare (Amare, 1987). However, the national average of bean yield in Ethiopia is very low (0.6 - 0.7 ton/ha). The major limiting factor for low yield is thought to be

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diseases (Habtu *et al*., 1996). Seedborne fungi are among the most important plant pathogens that cause direct and indirect losses of the bean crop throughout the world (Schwartz and Galvez, 1980; Hall, 1994; Abdelmonem, 2000). Losses are associated with high disease epidemic in the field consequently causes reduced seed yield and quality attributing to discoloration and blemish of seeds and green pods (Hellene, 1988). Moreover, seedborne pathogens play an important role for the dispersal and dissemination of several economically important diseases in the production field within the country and between countries (Agarwal and Sinclair, 1997). In a bean production system like in Ethiopia where there is no seed health and certification scheme, the risk of seed infection by seedborne fungi could be high.

Different types of fungal diseases are reported to cause damage on the bean plant in the field. Among others, bean anthracnose (*Colletotrichum lindemuthianum*), angular leaf spot (*Phaeoisariopsis griseola*), aschochyta blight (*Ascochyta phaseolorum*), ashy stem blight (*Macrophomina phaseolina*) are reported to be the major fungal pathogens causing foliar diseases of *Phaseolus* beans under field condition in Ethiopia (Habtu and Abiy, 1995, Habtu, *et al*., 1996). However, the association of these major fungal pathogens and other fungi with *Phaseolus* bean seeds has not been adequately investigated.

Survey and identification of plant pathogens is important to understand the association of pathogens with a specific host plant and to describe their geographic distribution (Agrios, 1997). Application of GIS on plant disease distribution map is helpful to store and refer spatially referenced point data (Nelson, *et al*., 1999). Mathur (1995) emphasised the need of surveys of seedborne pathogen by having good amounts of sample unit of different crop species and cultivars so that it enables researchers to set research priorities. Detail understanding of the type, occurrence, association with seed, and geographic distribution of major pathogens is a prerequisite to formulate rational integrated and sustainable disease management practices in different agro-ecologies. Therefore, the occurrence of major seedborne fungi of *Phaseolus* beans and their geographic distribution are needed to be investigated.

The objective of this study was to investigate the occurrence, and to map the geographic distribution of major seedborne fungal pathogens associated with *Phaseolus* bean seeds in various bean growing agro-ecological regions of Ethiopia.

MATERIALS AND METHODS

Survey areas and sample collection

Disease survey was carried out in the major bean growing regions of Ethiopia representing the lowlands (central rift valley), mid altitudes (north and southern part) and highlands (north-west and western part of the country) during the 2003 crop season. A total of 245 *Phaseolus* bean seed samples (172, 51, and 22) of common bean, climbing bean and green bean respectively were collected from the major bean-growing areas of the country. The seed samples were collected from various sources and cropping practices. These included farmers' fields, experimental fields, large-scale commercial farms and local markets. Geographic features like latitude, longitude and altitude were recorded from all surveyed areas using handheld Global Positioning System (GPS), "Garmen" with 12 channels to trace back the specific locations and types of seedborne fungi. All collected seed samples were kept at +5 °C in a refrigerator. The seed samples of different *Phaseolus* bean collected from various agro-ecologies were tested for seed-borne fungi by the standard blotter method (International Seed Testing Association, 1993) and agar plate method.

Detection of seedborne fungi using standard blotter method

Four hundred untreated and 1% sodium

hypochlorite pre-treated seeds were plated on four layers of water soaked blotter, 10 seeds per petri dish. The dishes were then incubated for seven days in a growth chamber SGC097.C with programmer, controller and sensor of combined temperature 24 ± 1°C and humidity probe under 95% humidity and 12 hours photoperiod of pre-programmed light conditions (Maden *et al*., 1975). The bottom of the compartment was filled with clean water covered by a glass plate. Slight air current was forced through a plastic pipe in the center at the bottom against the underside of the glass. Such high air humidity enabled the blotters remoistened during incubation and facilitated fungal sporulation. After seven days of incubation samples were examined using stereomicroscope with 20-30x magnification for the growth of fungi. The habit characters of various fungi associated with seeds was recorded. Further identification of fungi was made using compound microscope.

Detection of seedborne fungi using agar plate method

Seeds of Phaseolus bean treated with 1% sodium hypochlorite were plated on potato dextrose agar (PDA), and then incubated as it was done in the standard blotter method. Microscopic examination was made after seven days of incubation. Comparison on the efficiency of standard blotter and agar plate methods was also made by using naturally infected seed lots of common bean seed by the three major seed borne fungi (*C. lindemuthianum, A. phaseolorum,* and *P. griseola*).

Geographic distribution of major seedborne fungi of *phaseolus* beans

Important geographic features such as latitude, longitude, and altitude were recorded with the help of handheld Global Positioning System (GPS) “Garmen” with 12 channels. Satellite signals were received using the GPS and position of the coordinates was recorded. The collected coordinate information was downloaded into the computer and all necessary descriptive information were incorporated. Consequently, using the latitude and longitude coordinates the survey route and geographic distribution of major seedborne fungi maps were prepared using Geographic Information System (GIS) software (Arc view GIS) version 8.2.

RESULTS AND DISCUSSION

Detection and identification of seedborne fungi of *phaseolus* beans

Symptoms of seed infection by seedborne fungi varied depending on bean types and severity of infection. Common bean seeds with severe infection by *C. lindemuthianum* showed dark brown spot associated with characteristic symptoms of depressed sunken lesion both on pod and seed surface (Figure 3 C & D). When seed infection was not severe, it was difficult to differentiate symptoms of different pathogen by the naked eyes.

This study showed that *Phaseolus* bean seeds could be attacked by several economically important seedborne fungal pathogens. A total of thirteen seed-borne fungi of different genus was identified. Seed infection levels of *Phaseolus* bean with different seedborne fungi ranges between 0.2 - 14.5%. Bean seed infection levels varied between localities, growing conditions, and bean types. Seeds of common bean were more susceptible to all major seedborne fungal pathogens than climbing beans and green beans. High number of fungal microflora was also associated with common bean seeds (Table 1). The severity of seed infection by the major pathogens was very high on seeds collected from fields of small-scale farmer. This was mainly because small-scale farmers did not have the access to improve disease free seeds. Instead, they used to grow their own saved seeds from the previous harvests, without prior knowledge on the primary inoculum present in the seeds.

Bean anthracnose was found to be the most widespread with high percent seed infection. *C. lindemuthianum* was recovered from 26.2%, 19.6, and 13.6% of the seed samples of common bean, climbing bean and green bean respectively; whereas *P. griseola* was recovered from 18.6% and 15.7% of common bean and climbing bean respectively. *P. griseola* and *A. phaseolorum* were not detected from green bean seeds (Figure 1).

All these fungal pathogens are also economically important seedborne pathogens of beans in many parts of the world (Richardson, 1979). *C. lindemuthianum* is found to be the most important seedborne pathogen and is found in high frequency with severe crop damage followed by *P. griseola* and *A. phaseolorum* in many bean growing areas of Ethiopia. Chang *et al*., (2001) reported that several species of fungal microflora were isolated from naturally infected common bean seeds in Canada and *C. lindemuthianum* was found to be the most predominant fungus associated with bean seeds.

In the western part of Ethiopia, small-scale farmers grow climbing bean (*Phaseolus coccineus*) in their gardens. All three major seed borne fungi identified from common beans were also isolated from climbing beans and all the isolates proved to

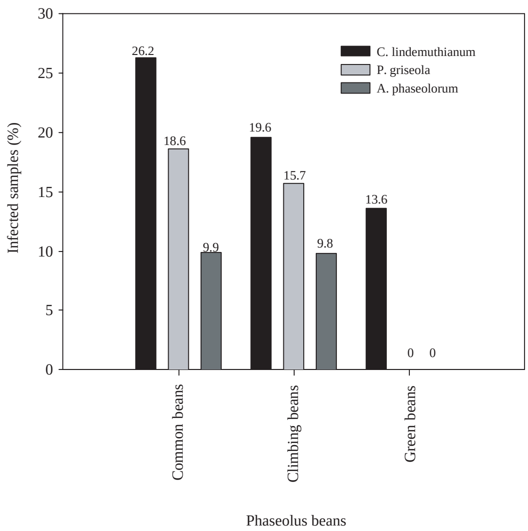

Figure 1 Infected samples (%) of *Phaseolus* bean seeds by major seedborne fungi during 2003 crop season.

Table 1 Numbers of infected seed sample and incidences (%) of seedborne fungi of common bean (*Phaseolus vulgaris)* seed obtained from different agro-ecologies of Ethiopia during 2003 crop season.

Fungi	Numbers of infected sample	Seed infection (%)	
		Range	Average
Colletotrichum lindemuthianum	45	1.2-14.5	8.4
Phaeoisariopsis griseola	32	0.5-12.3	5.6
Ascochyta phaseolorum	17	0.6-7.4	3.7
Macrophomina phaseoli	10	0.3-4.5	2.8
Fusarium moniliforme	3	0.2-2.5	0.8
Fusarium oxysporum	5	0.3-2.3	0.9
Aspergillus sp.	6	1.2-4.5	2.4
Botrytis sp.	1	0.3-2.3	0.4
Phoma exigua	4	0.4-2.6	1.0
Sclerotinia sp.	1	0.6	0.6
Trichotecium sp.	3	0.2-1.6	0.5
Alternaria alternta	4	0.4-1.2	1.3

attack *Phaseolus vulgaris*. Since climbing bean is a long duration crop and sometimes it can be grown throughout the year as a garden crop, it could serve as a primary source of inoculum of these major seed borne pathogens from season to season. Whereas, in the central rift valley of the country where 60% of the country's common bean production is grown (Aleligne, 1990), green beans are cultivated in state owned large commercial farms and also private investors under irrigation in continuous planting throughout the year. This type of cropping system also creates favourable condition for the survival and dissemination of important seed borne pathogens such as *C. lindemuthianum*. Noitalics rot of beans which is caused by *Phytophthora* sp. was detected from green bean pods and immature seeds collected from commercial bean farms in the central rift valley. This disease is common and causes damage to the bean plant in the South and Central America (Hall, 1994). In this study, *Phytophthora* rot of beans caused by *Phytophthora* sp. was reported for the first time in Ethiopia.

Detection of major seedborne pathogens using the standard blotter method gave the higher amount of seed infection ($P < 0.01$) as compared to the agar plate method (Figure 4). This might be due to the availability of free moisture on the moist blotter papers than the agar plates. The bean seed coat absorbed more water from the wet blotters and enabled the fungi to easily grow and sporulate, whereas on the agar plates, the bean seeds became dry and sporulation of the fungus reduced as compared to the standard blotter method. Blotter method was the easiest, efficient and economical detection technique for *Colletotrichum lindemuthianum*, *Ascochyta phaseolorum*, and *Phaeoisariopsis griseola* from naturally infected *Phaseolus* bean seeds.

Geographic distribution of major seedborne fungi of *Phaseolus* beans

A total of thirteen seedborne fungi of different genera were identified from samples collected from various bean growing agro-ecologies of Ethiopia (Table 1 & 2). Seed infection of individual samples by different seedborne fungi ranged from 0.2-14.5% and the most widespread

Table 2 Number of infected seed samples and infection percentage of climbing bean and green bean seeds by different seedborne fungi obtained from different agro-ecologies of Ethiopia during 2003 crop season.

Fungi	Number of infected samples		Seed infection (%)			
			Range		Average	
	Climbing bean	Green bean	Climbing bean	Green bean	Climbing bean	Green bean
Colletotrichum lindemuthianum	10	3	2.0 - 8.6	1.0 - 4.2	4.8	2.6
Phaeoisariopsis griseola	8	0	0.8 - 10.4	0	5.2	0
Ascochyta phaseolorum	5	0	1.1 - 6.4	0	3.2	0
Macrophomina phaseolina	4	0	0.4 - 2.4	0	1.2	0
Fusarium moniliforme	2	1	0.4 -1.8	0.9	1.0	0
Aspergillus sp.	4	2	1.6 - 5.8	1.1 - 2.6	3.2	1.4
Botrytis sp.	0	2	0	1.0 - 2.8	0	1.6
Tricotecium sp.	2	0	1.2 - 2.6	0	1.8	0
Phytophthora sp.	0	6	0	1.5 - 4.8	0	3.1
Alternaria alternata	6	2	1.8 - 6.5	1.0 - 2.3	3.4	1.7

seedborne fungi of *Phaseolus* beans were *C. lindemuthianum*, *P. griseola*, and *A. phaseolorum*. These pathogens attack major *Phaseolus* beans and widely distributed in many of bean growing areas of Ethiopia. The south, west, and north-western part of the country is characterized with high rainfall, whereas the central and eastern part get low and erratic rainfall. However, during the 2003 crop season, there was high rainfall in all parts of the country. This created favourable condition for the development of some of the seedborne pathogens even in the semi-arid areas. In the western part of the country, where there is high and frequent rainfall, all these pathogens were found simultaneously in the same area or bean field (Figure. 2). Bean anthracnose was the most widespread and predominant fungal pathogen in almost all bean growing areas of the country including the lowland arid areas where there was low and erratic rainfall during the crop season. Even though there was field infection of beans by bean anthracnose in the semi arid areas of the central rift valley, seed infection by *C. lindemuthianum* was very low. This might be due to the late appearance of the disease in the field and unfavourable environmental conditions during the active growth stage of the bean plant. Angular leaf spot and ascochyta blight were more abundant in areas with high rainfall and humidity particularly to the southern and western part of Ethiopia such as Ambo, Bako, Pawe, Areka and Jimma area (Figure 2). These fungi were not detected from seeds collected from the central rift valley where the bulk of dry common bean production is found (Table 3).

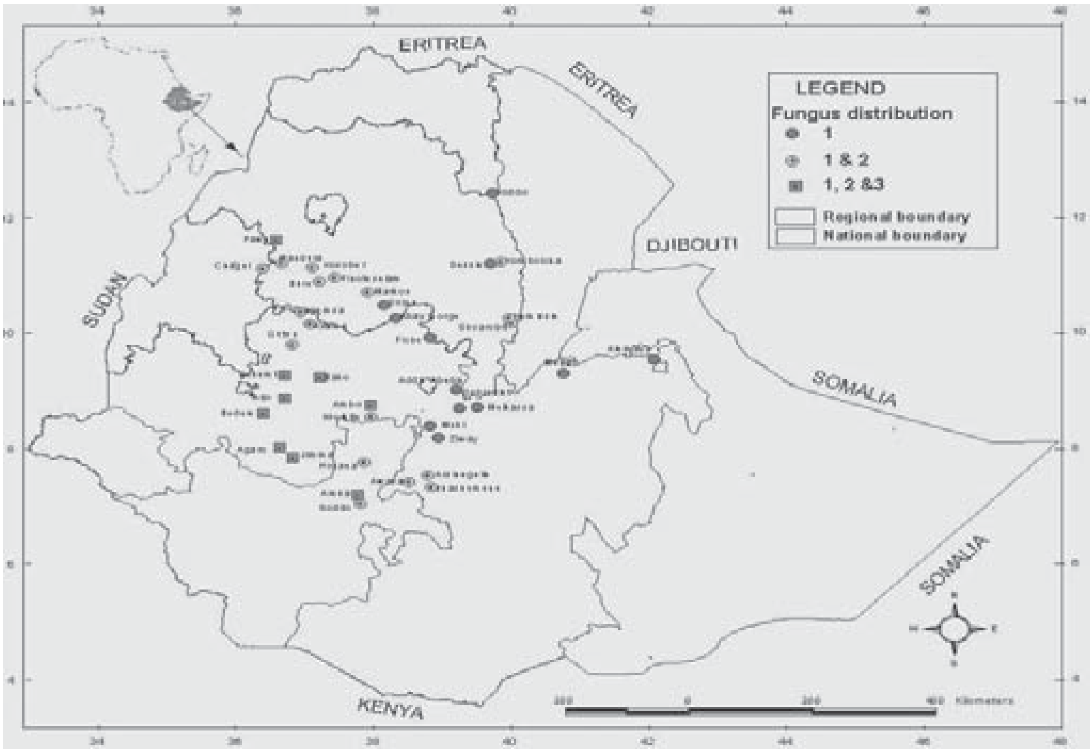

Figure 2 Geographic distribution of major seedborne fungi of *Phaseolus* beans in Ethiopia during the 2003 main crop season (**1**- *Colletotrichum lindemuthianum*, **2**-*Phaeoisariopsis griseola*, **3**-*Ascochyta phaseolorum*).

Table 3 Geographic distribution and severity of major seed borne fungi of common bean at different bean growing areas during 2003 crop season.

Location	Geographic locations	Altitude (M.a.s.l)	Severity of major seedborne fungi		
			C. lindemuthianum	*P. griseola*	*A. phaseolorum*
Melkassa	39 °.50' E 8 °.60' N	1650	++	-	-
Debrezeit	39 °.19' E 8 °.91' N	1940	++	-	-
Dejen	38 °.15' E 10 °.37' N	2476	+	+	-
Markos	37 °.91' E 10 °.58' N	2470	++	+	-
Finoteselam	37 °.43' E 10 °.84' N	1875	++	++	-
Chagni[1]	37 °.58' E 11 °.01' N	1665	++	++	-
Pawe[1]	36 °.60' E 11 °.50' N	1126	++	+++	+
Bure[1]	37 °.21' E 10 °.77' N	2150	++	+++	-
Guten	36 °.81' E 9 °.68'N	1404	+	+	-
Nekemt[1]	36 °.71' E 9 °.14' N	2130	+++	+++	++
Bako[1]	37 °.23' E 9 °.11' N	1650	+++	+++	+++
Ambo[1]	37 °.86' E 8 °.64' N	2200	+++	+++	+++
Arjo[1]	36 °.71' E 8 °.75' N	2526	++	+	+
Bedele[1]	36 °.40' E 8 °.50' N	2027	++	++	+
Agaro[1]	36 °.64' E 7 °.89' N	1684	++	+++	+
Jimma[1]	38 °.88' E 7 °.74' N	1750	++	+++	++
Wolkite[1]	37 °.96' E 8 °.42' N	1845	++	++	-
Areka[1]	37 °.78' E 7 °.08' N	1768	++	+++	+
Sodo[1]	37 °.81' E 6 °.92' N	1850	++	+++	-
Awassa[1]	38 °.52' E 7 °.30' N	1730	++	+++	-
Shashemene	38 °.51' E 7 °.33'N	1940	++	+	-
Arssinegelle	38 °.79' E 7 °.41' N	1960	++	+	-
Ziway	38 °.94' E 8 °.06' N	1660	++	+	-
Meki	38 °.82' E 8 °.27' N	1675	+	+	-
Meisso	40 °.76' E 9 °.19' N	1350	+	+	-
Alemaya	42 °.27' E 9 °.43' N	2050	+++	-	-
Shoarobit	40 °.00' E10 °.06' N	1260	++	+	-
Kemissie	39 °.97' E 10 °.14' N	1780	++	+	-
Kombolcha	39 °.85' E 11 °.11' N	1900	++	++	-
Dessie	39 °.70' E 11 °.08' N	1950	++	+	-
Kobbo	39 °.72' E 12 °.30' N	1450	++	-	-

[1] - Areas with high rainfall

- = No disease; + = Slight; ++ = Moderate; +++ = Severe

M.a.s.l. - Meters above sea level

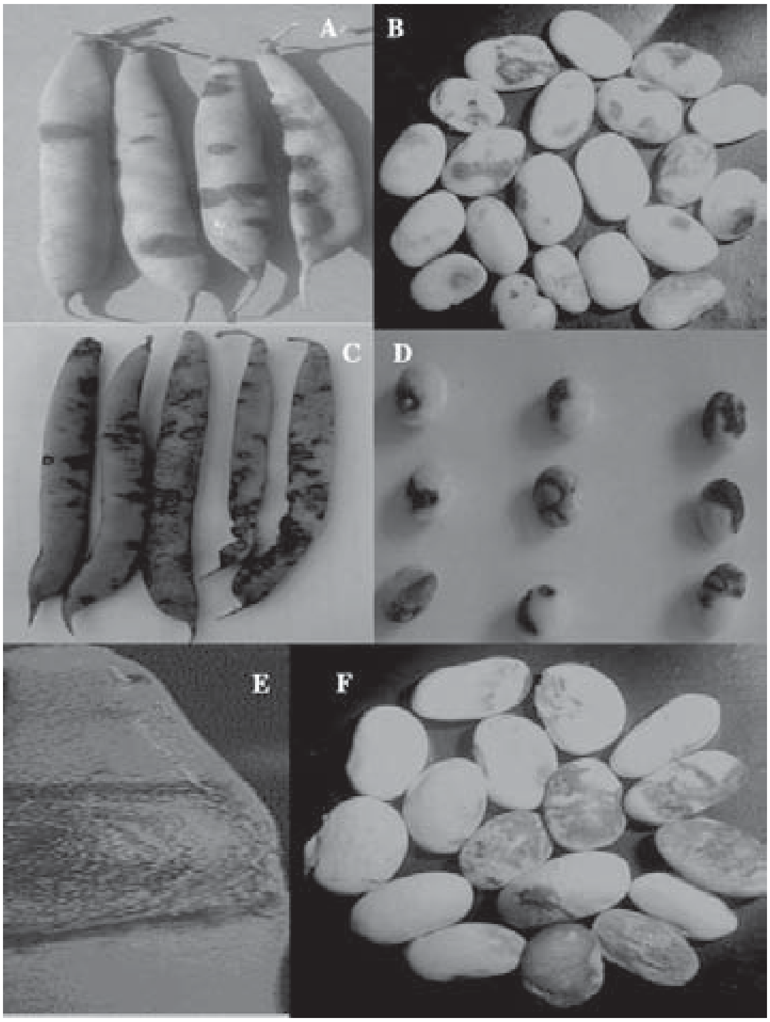

Figure 3 Symptoms of major seedborne fungal diseases under field condition on pods and seeds of common beans, *Paseolus vulgaris L.* (A & B- Angular leaf spot, C & D-Anthracnose, E & F-Ascochyta blight).

CONCLUSION

Basic information on the occurrences and geographic distribution of major plant disease, and their association with seeds is very important for setting research priorities for further disease management strategies in different agro-ecologies. From this study, it could be concluded that several seed borne fungi were highly associated with bean seeds. The majority of fungi identified in this study were known to be seed transmitted and caused heavy crop loss in different bean growing parts of the world. The presence of different seed borne pathogens in bean seeds warrants for research attention in the area of seed pathology. Seed certification schemes must be strengthen during production, and release of improved bean varieties to the growers. Infection of bean seeds by large number of seedborne fungi also implies the importance of inspecting seed lots of *Phaseolus* beans for potential seedborne pathogens in local germplasm maintenance, introduction or exchange

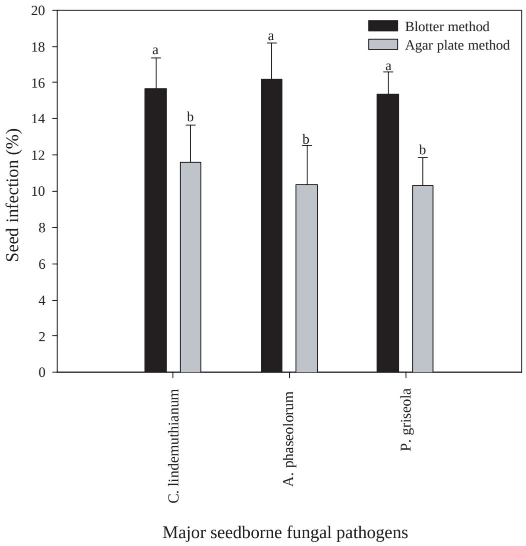

Figure 4 Comparison of standard blotter and agar plate methods for the detection of seedborne fungi using naturally infected common bean seeds.

between institutions and countries. Since there is a change or shift in types and occurrence of plant pathogens in different agro-ecologies, routine disease surveys and identification work is important for better understanding of fungal myco-flora associated with *Phaseolus* bean seeds. Distribution maps along with illustrations of the spread and spatial patterns of diseases across regions should be emphasised.

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