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SEED QUALITY AND MYCOFLORA ASSOCIATED WITH CHICKPEA (*Cicer arientinum* L.) SEED IN ETHIOPIA

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ABSTRACT

The study was conducted at Ethiopian Institute of Agricultural Research, Haramaya University and Jimma University, Ethiopia. Crop yield is directly associated with the physical and biological quality of planting material or seed. Ninety-nine (99) chickpea seed lot samples were collected for physical purity, seed health and germination tests from major chickpea growing areas in Ethiopia in the 2016/17 cropping season to assess the status of chickpea seed health and quality among subsistence farmers, research stations and seed growers. The seed lots were grouped as researcher saved, farmers saved and seed growers' saved seed. The maximum physical purity of 97.5% was recorded for the researcher saved seed lots, 90.8% for the seed growers and 87.4 % for the farmers saved seeds. Foreign matters and broken seeds were the most contaminants found in the seed lots. The seed germination percentages were in the range of 96.3% to 98.5% for all seed sources and there were no significant differences among the seed lot samples. A total of seventeen (17) fungi species were isolated from all seed sources with different frequency and amount. These are *Fusarium* spp*., Aspergillus* sp*., A. niger, A. flavus, A. nidulans, A. candidus, A. fumigatus, Penicillium* sp*., Rhizopus* sp*., Verticillium* sp*., Rhizoctonia* sp*., Pythium* sp*., Alternaria* sp*., Helminthosporium* sp*. Phylostica* sp*., Cladosporium* sp*., Negrospora* sp*. Aspergillus flavus* was found the most dominant with recovery (Relative Density=21.53%, Infection rate=10.36%, and Infection Frequency=25.59 %) from all seed lots. There were high variations in relative density, Infection rate, and Infection frequency among isolated fungi. *Ascochyta rabiei* the most important chickpea disease was not found in this study. This might relate to the incidence and prevalence of sample collection season/cropping year which was low in expected areas. The current study concluded that there are seed qualities and seed health management issues with regards to different seed sources (farmers, research and private sectors); this entails strong seed quality control and growers' awareness creation on storage sanitation, seed health test before sowing, and production of healthy crops. To keep the seed health in a better condition, seed growers should keep a wider interval of rotation, develop use of the healthy improved seed, after some generation (4-5), seed grading to avoid loss of physical purity, use of appropriate storage container (ventilated and clean), seed dressing with safe pesticides, and appropriate moisture level for storage (about 14%) should have to keep. Longer storage also gives a chance to contaminate the whole seed and can expose to decay.

Key words: Chickpea, fungi, germination, mycoflora, purity, seed health, seed sources

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INTRODUCTION

A healthy seed is an assurance and proxy of crop establishment for sustainable productivity. As ninety percent of the food crops are grown from seed, it is a basic and vital input to improve agricultural productivity and production [1, 2]. Since 2000, the global seed market has been increasing its industry dramatically in both conventional and genetically modified seeds reaching approximately US $51 billion in 2014. Europe's seed market in 2012 reached around €7 billion corresponding to 20% of the global market ranking third after the USA and China [3]. Results of seed health tests in the world shows seed can be infected by many serious seed-borne diseases and uncontrolled cross-boundary movement can result in epidemics in its destination countries. Historically, infected seeds were responsible for the introduction of Ascochyta blight of chickpea into Australia [4], Canada [5], Iran [6], and the USA [7, 8].

Seed health can also affect seed movement/trade between regions. Seed producers are currently using different mechanisms such as chemical, biotech, and physical seed invigoration alternatively to secure healthy seed and good crop stand. However, seed health management is the cheap method of controlling seed borne pests.

According to Abebe [9] and Abebe *et al.* [10], the seed sector is poorly developed in Ethiopia and different seed production systems exist parallel to one another. These are: informal seed systems (seed grower associations, farmer's unions, individual farmers and the likes), community-based seed systems (group or individual farmers who grow improved seed for seed purpose as a business), and formal seed systems (government-supported commercial or private enterprise seed system). Bishaw *et al*. [11], reported 80-90% of major crops seed demand in Ethiopia was fulfilled by the informal sector, no exact figures were available for the percent share of the formal system and community-based seed system. The formal sector supplies less than 2% of the chickpea seed requirement in Ethiopia [12]. Cereals, mainly hybrid maize, wheat and sorghum are the major seed produced and supplied by the Ethiopian Seed Enterprise (ESE), which is government owned company. Consequently, the seed demand for legume crops, including chickpea, is yet unaddressed [13].

Seed-borne diseases are among the most important biotic constraints for both pre- and post-emergence death of plants, affect seedling vigor, and cause a reduction in germination, poor crop stand and variation in plant morphology [14, 15, 16]. Many fungal species viz., *Alternaria porri, A. alternata, Aspergillus amstelodami, A. flavus, A. fumigatus, A. nidulans, A. niger, A. sydowi*, *A. wentii, Botrytis cinerea, Cladosporium macrocarpum, Curvularia lunata, Fusarium equiseti, F. moniliforme, F. oxysporum, F. semitectum, Macrophomina phaseolina, Myrothecium roridum, Penicillium crustosum , Rhizoctonia sp*., and *Rhizopus arrhizus* have been reported from chickpea globally [17, 18, 19, 20, 21]. Alemu *et al*. [20] reported 15 fungal and one bacterium from chickpea seed assay in Ethiopia with *Penicillium* sp. and *A. flavus* having the highest recovery and being the most important. Seed borne micro-organisms were reported to cause lower physical quality and germination level, high disease epidemic and poor crop stand [12]. The infected seeds may fail to germinate, transmit disease from seed to seedling and from seedling to growing plants [22].

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Empirical information regarding chickpea seed quality and health particularly under subsistence farmers and commercial seed farms in Ethiopia has not been reported. It was hypothesized that different seed sources and the way they are managed could influence the status of chickpea seed quality and health. Assessing seed quality and health is critically important for improving the production and productivity of chickpea; besides, it is critical steps towards developing sustainable integrated chickpea disease management. There is insufficient or no updated information on the current chickpea seed health status in Ethiopia, one of the major producers of chickpea globally http://www.fao.org/faostat/en/#home December 2019. The objective of the present study was to assess the status of chickpea seed health and quality among subsistence farmers, research stations and seed growers in Ethiopia. This paper, therefore, reports on the current chickpea seed quality and health from major chickpea seed sources and production areas of Ethiopia with implications to chickpea productivity and disease management.

MATERIALS AND METHODS

Sampling

Ninety-nine (99) samples for physical purity and 76 chickpea seed samples for seed health and germination were randomly collected from the major seed sources (farmers saved seeds, seed growers and Research stations) in different parts of chickpea growing areas form 2016/17 harvest. The sampling includes ten districts in two regional states where chickpea is grown as major pulse crop and covers large area in Ethiopia: Oromia region – East Shoa zone **(**Ada'a district, Gimbichu district, Akaki district and Lume district); Amhara region - North Shoa zone (Minjar shenkora district), Gonder **(**East Dembia district, West Dembia district, East Belesa and West Belesa districts) and East Gojam (Dejen district) (Fig 1 and Table 1). Districts of each region and research were purposely selected and individual farmers were taken randomly. The chickpea varieties were both Kabuli and Desi type and harvest of similar season. Chickpea seed growers for business purpose around Debre-zeit were purposely selected and samples were collected. Basic seeds were sampled from Debre-ziet Research Center, Gonder Research Centers and Ambo Plant Protection Research Center.

About 500g of seed lot was collected per sample and maintained at Pulse Pathology Laboratory at Ethiopian Institute of Agricultural Research (EIAR) Debre Zeit Agricultural Research Center Pulse Pathology Laboratory and kept 25 ± 2^0C for sixty days until processing. The sample diagnoses were carried out at Haramaya University, Plant Pathology Laboratory and Debre Zeit Agricultural Research Center, Ethiopia.

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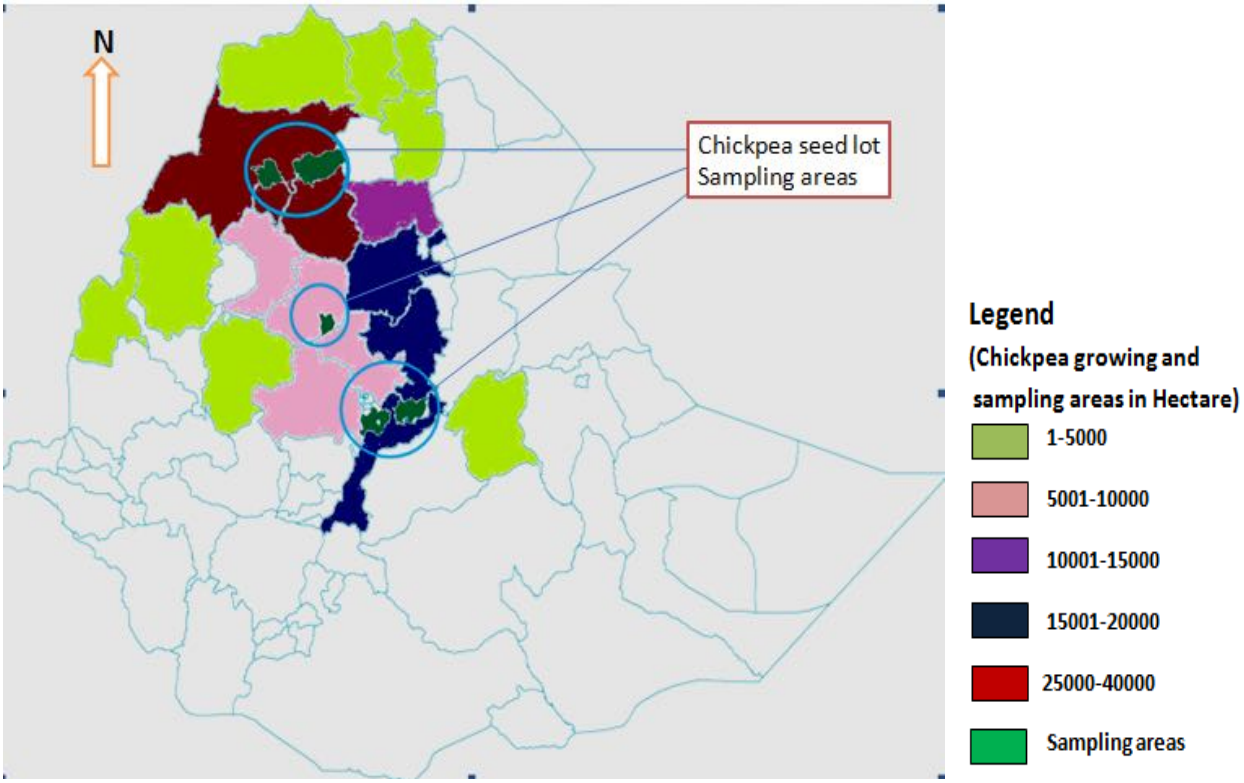

Figure 1: Chickpea production distribution (Central Statistical Agency of Ethiopia 2016/5/16) and sample areas map

Physical purity

Five hundred grams of sampled chickpea seeds were taken and separated according to the physical health status. Hence the foreign matter and healthy seed were visually separated and their weight is taken with a digital balance (Denver Instrument company, XL-1810).

Germination test

From 500g sample, a sub-sample of two hundred seeds from each sample were taken and placed on three layers of moistened blotters in 12cm diameter Petri-plates at the rate of 25seeds/ Petri-plate. International Seed Testing Association (ISTA) techniques [23] were followed. Plates were then incubated for 7 days. Germination percent was recorded as:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds used}} \times 100$$

Seed mycoflora

Fifteen (15) seeds of test samples were placed onto the 12 cm radius Petri-plates at equidistance under aseptic condition. The test samples were set in fourteen replications each with fifteen seed per Petri-plates and kept in an incubator at 27°C for a maximum of ten days. The growths of microflora were inspected every two days and sub-cultured until the eleventh day. The seeds were examined under a microscope for the determination of seed mycoflora. The seed-borne fungi found on each seed were isolated

and identified using standard microbiological methods according to ISTA techniques [23] and brought into pure cultures and maintained on PDA (Potato Dextrose Agar) slants. Light microscopy was used for identification based on morphological characters of mycelia structure, form of colony growth and fruiting bodies according to Barnett, H.L. [24], Maren [25] and Watanabe [26] illustrations.

Data analysis:
Frequency of isolation of fungi (%), relative density of isolated fungi (%) and incidence of fungi (%) were recorded and calculated.

$$\text{IF (\%)} = \frac{\text{No. of samples of occurrence of a species}}{\text{Total No. of samples}} \times 100$$

Where: IF = Isolation frequency

$$\text{RD (\%)} = \frac{\text{No. of isolated genus or species}}{\text{Total No. of isolated fungi}} \times 100$$

Where: RD= Relative density

$$\text{IN \%} = \frac{\text{Number of infected seeds}}{\text{Total number of seeds}} \times 100$$

Where: IN = Incidence of fungi

Analysis of variance (ANOVA) was performed using general linear model (GLM) procedure and means were separated using Tukey's Studentized Range (HSD) Test at significance level of alpha 5% (SAS Institute inc. Cary NC 27513 USA, 2002 software).

RESULTS AND DISCUSSION

Physical purity and germination test
There were significant differences ($p<0.001$) between the three seed sources in their physical purity (Table 2). Chickpea seeds from research stations had the highest physical purity (97.5%) followed by commercial seed growers (90.8%) then seed from farmers (87.4%) (Table 2). There were no differences between samples of each seed lots per seed source group. The level of seed germination ranged from 96% to 98% among the three seed categories and there was no significant difference between seed sources (Figure 2). Farmer-saved seeds were highly contaminated with broken seed and foreign matters than seeds from research centers and from commercial growers. Next to microflora infestation, information about the physical purity of seed is probably the second most important factor/parameter for seed health that ensures future crop stand. The use of poor-quality seed can directly affect the seed rate that results in poor crop stands in the field. It is estimated that about 20-25% yield increments can be achieved through the use of quality seed to the improved varieties [27].

Farmers' seed lots were mostly contaminated by foreign matters (sand, soil debris, broken seeds, shriveled seeds, and other seeds). Broken seeds were mainly by insect

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damage in the field that could be pod borer, the most important pest in all chickpea growing areas and during threshing [28, 29].

Community (commercial) seed growers are the owners of improved varieties where they became seed sources for local farmers and sometimes sell to Government and Non-government institutions. Though seed growers' field and seed are inspected by affiliated groups, the amount of inert matter is almost close to farmers saved seed (90.8% and 87.4%; respectively) (Table 2). These foreign matters contamination could be introduced mainly during threshing which is usually undertaken on the bare field that creates conducive environment of mixing of same size inert matters.

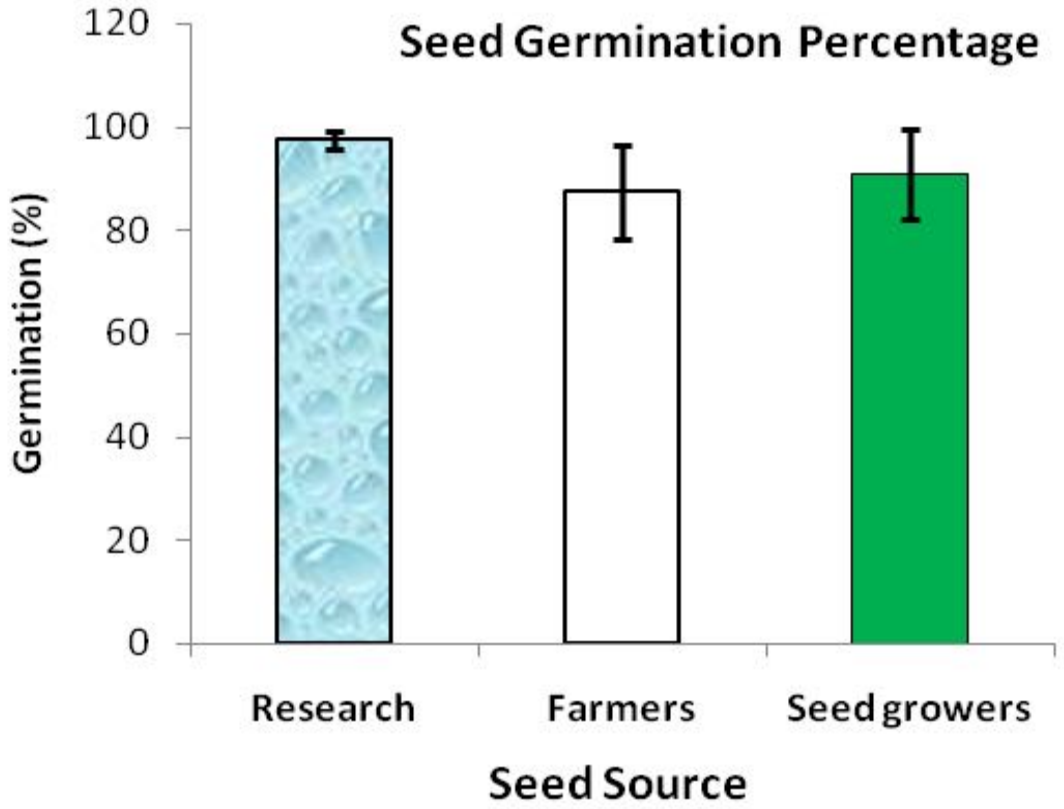

Figure 2: Chickpea seed germination rate of different seed sources in 2016/17 cropping season

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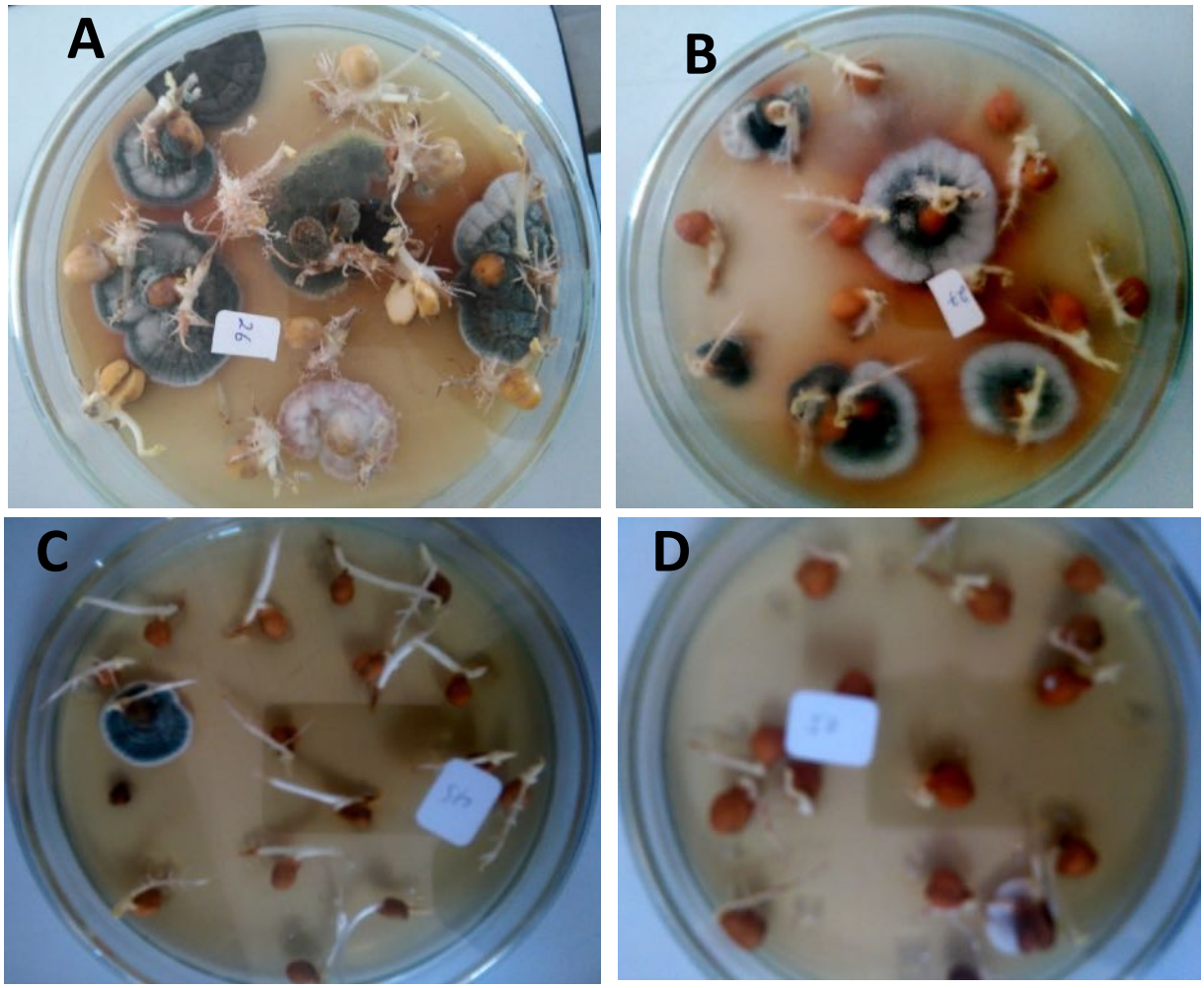

Figure 3: **Mycoflora developed from seed lots A&B) highly infected seed C&D) seeds with Lower infestation/infection**

Mycoflora associated with chickpea seed

About seventeen (17) fungal species belonging to eleven genera were isolated from all samples collected based on morphological characters of mycelia structure, form of colony growth and fruiting bodies (Table 3) (Figure 3). Most of the seed samples were infected with a range of fungal species: *Fusarium* spp., *Aspergillus* sp., *A. niger*, *A. flavus*, *A. nidulan, A. candidus, A. fumigatus, Penicillium crustosum*, *Rhizopus* sp., *Verticillium* sp., *Rhizoctonia* sp., *Pythium* sp., *A. alternata*., *Helminthosporium* sp., *Phylostica* sp., *Cladosporium* sp. and *Negrospora* sp.

Out of 17 species of mycoflora recovered from the current test, 11 were common for all seed samples. In general, except infection frequency, *Aspergillus flavus* were the most common fungi recorded (RD=21.53%, IN=10.36% and IF=25.59 %) among isolated fungi and followed by *Penicillium crustosum* (RD: 18.35%, IN: 5.80% and IF: 26.11%), *Fusarium* sp. (RD: 12.87%, IN: 4.35% and IF: 23.37 %) and *Aspergillus* sp. (RD: 12.58%, IN: 3.85% and IF: 18.45%) (Table 3). These fungi were still dominant and

similar across all the seed sources (Table 4). The percent relative density, infection rate and infection frequency of isolated fungi were statistically different ($p<0.0001$) (Table 4) but it was not statistically different among seed sources (data not presented here). Other genera isolated as significant components of the mycoflora included but no significant difference between the species (Table 3 and 4). Either internally or externally seed associated pathogens, seed-borne microflora are the important determinant of seed quality [30, 31]. Many foliar and root chickpeas seed-borne diseases were reported from Ethiopia and about six of them were major and reported as production constraints [32]. The Ethiopian research system has released twenty-six chickpea varieties [33]; nevertheless, seed quality and health are an area that still needs improvements.

A. flavus, *Penicillium crustosum* and the most important seed and soil born pathogen, *Fusarium* sp., were significant in all tested seed sources. These fungi were reported by different authors and were not new to chickpea crop and similar pathogens were recovered from chickpea and other crop storage. Among these pathogens; *Aspergillus* sp. and *Penicillium* sp. can cause seed deterioration and loss of its viability [17, 18, 34, 35, 36, 37]. The results of the current study agree with the research reported four decades ago in Ethiopia [20]. The common fungal seed mycoflora; *Aspergillus* and *Penicillium* are mostly known to produce mycotoxins that adversely affect the seed germination, shoot and root length of all test pulses in variable quantity [38]. From the previous study, toxins of the seed-borne fungi were responsible for; inhibition of normal growth of seedlings in different crops, germination failure, mycotoxin production and permanent contamination of soil [19, 35, 39].

Significant protein losses were reported from seeds of black gram, green gram, wheat, maize and barley due to some seed-borne diseases like *A. flavus*, *F. semitectum* and *F. oxysporum* under different storage conditions [19, 40]. It depends, however, on different factors whether the infected seed results in germination failure or cause of disease on growing seedlings [35, 36]. *Aspergillus* sp., in general, out-numbered all the other pathogens and widely distributed in the seed samples tested. The fast growth of *Aspergillus* sp. may inhibit the growth and detection of the slow-growing pathogens present internally like ascochyta blight [41]. *A. flavus* was known mycotoxin producer and produce aflatoxin B1, B2, G1 and G2 which are carcinogenic (hepatocarcinogenic) [42, 43]. *Rhizoctonia* sp., the causal agent of root rot and dry rot of chickpea were also significantly detected from Ethiopian chickpea seed. According to Pande *et al*. [44], root rot was common in warm dry climates and generally appears during late flowering and podding stages and can cause complete drying of chickpea plant.

Germination of all seed sources was high and not significantly different. Seed quality and health can influence seed germination in the field that determines the crop stand. This result might not be inclusive to detect the whole expected major chickpea diseases rather it focuses on some fungal pathogens which can grow on the general medium. Though different strategies have been taken to minimize wilt diseases in Ethiopia, the scenario still is increasing in the field [45]. This might be associated with the land use system, that farmers follow short- rotation where the pathogen can survive in the soil for about six years [37]. Crop field performance at large depends on the health status of seed

but the presence of the pathogen in association with seed would not mean necessarily produce an unhealthy seedling.

CONCLUSION

In Ethiopia, most farmers are using double of the recommended seed rate (personal observation). This could be associated with seed health which is expected to affect healthy seedling. Longer storage also gives a chance to contaminate the whole seed and can expose to decay. In this study *Ascochyta rabiei* the most important chickpea disease was not found. This might relate to the incidence and prevalence of sample collection season/cropping year which was low in expected areas.

To keep the seed health in a better condition, seed growers should keep a wider interval of rotation, develop use of the healthy improved seed, after some generation (4-5), seed grading to avoid loss of physical purity, use of appropriate storage container (ventilated and clean), seed dressing with safe pesticides, and appropriate moisture level for storage (about 14%) should have to keep. The current study concludes that there are seed qualities and seed health management issues with regards to different seed sources (farmers, research and private sector); this entails strong seed quality control and growers' awareness creation on storage sanitation, seed health test before sowing, and production of healthy crops.

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Table 1: Chickpea seed collection sites in Ethiopia from 2016/17 harvest

Regional States	Zone	Destrict	Geographic Position System (GPS)
Oromia	East Shoa	Ade'a	39° 2' E 8° 40' N
		Gimbichu	39° 7' E 8° 58' N
		Akaki	38° 35'E 8° 51' N
		Lume	39° 15' E 8° 46' N
Amhara	North shoa	Minjar	39° 27' E 8° 54' N
	East Gojam	Dejen	38° 9' E 10° 16' N
	North Gonder	East Belesa	38° 19' E 12° 54' N
		West Belesa	37° 49' E 12° 28' N
		East Dembia	37° 19' E 12° 25' N
		West Dembia	37° 15' E 12° 21' N

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Table 2: Chickpea seed physical purity result collected from research centers, farmers and commercial seed growers in Ethiopia in 2016/2017

S.no.	Researchers saved seed purity %	Farmers seed purity%	Commercial seed growers seed purity%
1	98.4	84.7	88.0
2	98.4	86.5	87.2
3	95.8	94.2	96.7
4	95.9	77.6	96.0
5	91.1	85.8	100.0
6	91.0	99.0	100.0
7	99.2	87.5	94.8
8	99.2	92.7	94.1
9	95.5	47.1	82.5
10	95.3	91.9	83.8
11	97.3	97.6	98.0
12	97.4	91.7	97.7
13	95.8	90.0	95.2
14	96.0	87.9	95.3
15	95.1	94.0	98.9
16	94.9	88.0	98.5
17	98.3	95.0	81.4
18	98.3	92.0	56.7
19	100.0	96.0	98.0
20	100.0	95.3	98.0
21	98.5	80.3	62.3
22	98.5	92.0	59.2
23	99.0	95.9	85.0
24	99.0	99.0	80.0
25	100.0	94.0	97.1
26	98.0	96.0	97.0
27	98.0	97.2	97.7
28	100.0	78.1	97.6
29	99.0	88.7	91.3
30	99.0	95.6	100.0
31	99.0	65.0	98.5
32	100.0	94.4	95.9
33	98.0	32.7	94.0
Sum	3218.8	2883.6	2996.3
Mean	97.5a	87.4b	90.8b
Alpha 0.05	$p<0.001$		

Means with the same letter between groups are not significantly different

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Table 3: Fungi isolated from chickpea seed of different seed sources Ethiopia in 2016/17 cropping season

Isolated fungi	Relative Density %	Infection rate %	Infection Frequency %
Aspergillus flavus	3.41 (21.53 ± 24.88) a	2.30 (10.36 ±17.97) a	3.74 (25.59 ±28.90) a
Penicillium crustosum	3.16 (18.35 ± 24.07) ba	1.88 (5.80 ±7.79) ba	3.93 (26.11 ±28.38) a
Fusarium sp.	2.60 (12.87 ± 17.92) ba	1.62 (4.35 ±5.90) b	3.67 (23.37 ± 26.53) a
Aspergillus sp.	2.23 (12.58 ± 21.72) b	1.30(3.85 ± 6.44) b	2.89 (18.45 ±25.99) a
A. niger	0.91 (4.53 ± 12.29) c	0.58 (1.95 ±6.88) c	1.10 (6.10 ± 12.85) b
Rhizopus sp	1.07 (6.57 ± 16.98) c	0.63 (2.12 ±6.60) c	1.02 (6.28 ± 14.32) b
Pythium,	0.97 (6.59 ± 20.20) c	0.50 (1.61 ±5.57) c	0.93 (5.93 ±14.37) b
Rhizoctonia	0.71 (3.91 ± 12.85) c	0.48 (1.76 ±6.10) c	0.83 (5.58 ±13.68) b
A. nidulan	0.09 (0.66 ± 5.73) c	0.04 (0.12 ±1.02) c	0.21 (1.10 ± 6.85) b
A.candidus	0.05 (0.22 ± 1.91) c	0.03 (0.06 ±0.51) c	0.37 (0.99 ± 6.08) b
A. fumigatus	0.26 (1.96 ± 11.39) c	0.12 (0.42 ±2.46) c	0.21 (1.52 ± 6.94) b
Verticillium sp.	0.18 (0.55 ± 2.63) c	0.14 (0.22 ±0.97) c	0.30 (2.13 ± 8.28) b
Alternaria alternata	0.46 (2.16 ± 9.49) c	0.24 (0.53 ±1.93) c	0.52 (2.83 ± 8.33) b
Helminthosporium sp.	0.06 (0.16 ± 1.03) c	0.04 (0.05 ±0.34) c	0.08 (0.90 ± 5.89) b
Phylostica sp.	0.03 (0.06 ± 0.52) c	0.02 (0.01 ±0.13) c	0.05 (1.20 ± 6.62) b
Clamydosporium sp.	0.26 (0.80 ± 3.12) c	0.16 (0.27 ±0.97) c	0.38 (2.33 ± 7.95) b
Negrospora sp.	0.52 (2.57 ± 8.58) c	0.31 (0.97 ±3.89) c	0.61 (4.04 ±11.48) b

Original data square root transformed
Numbers in the parentheses are (Original data ± standard deviation)
Means with the same letter in a column are not significantly different

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Table 4: Fungi isolated from chickpea seed of different seed sources in Ethiopia in 2016/17 cropping season

Isolated Fungi	Farmers			Seed growers			Research stations		
	RD%	IN%	IF%	RD%	IN%	IF%	RD%	IN%	IF%
Aspergillus flavus	3.46a	2.44a	3.75a	3.46a	1.98ba	3.52bac	3.19a	2.04a	3.91a
Penicillium crustosum	3.32a	1.94ab	3.97a	2.80ba	1.90ba	3.99ba	2.88ba	1.62ba	3.73a
Fusarium sp.	2.63a	1.60b	3.64a	3.30a	2.14a	5.04a	1.88bac	1.19ba	2.50ba
Aspergillus sp.	2.56a	1.48b	3.11a	2.60bac	1.52bac	3.50bac	0.63bac	0.44ba	1.28 ba
A. niger	0.91b	0.58c	0.93b	0.18d	0.12c	0.34dc	1.61bac	0.99ba	2.40ba
Rhizopus sp	0.79b	0.44c	0.71b	1.15bdac	0.76bac	1.44bdc	2.08bac	1.21ba	1.81ba
Pythium,	0.70b	0.30c	0.60b	0.65bdc	0.37c	0.59dc	2.35bac	1.40ba	2.50ba
Rhizoctonia	0.42b	0.28c	0.55b	0.62bdc	0.27c	0.75dc	2.11bac	1.42ba	2.01ba
A. nidulan	0 .14b	0.06c	0.11b	0.00d	0.00c	0.83 bdc	0.00c	0.00b	0.00b
A.candidus	0.00b	0.00c	0.28b	0.00d	0.00c	0.83bdc	0.31bc	0.16b	0.31b
A. fumigatus	0.32b	0.14c	0.19b	0.00d	0.00c	0.00 d	0.28bc	0.18b	0.46b
Verticillium	0.12b	0.08c	0.23b	0.43bdc	0.54bc	0.94bdc	0.00c	0.00b	0.00b
Alternaria	0.49b	0.26c	0.51b	0.26dc	0.11c	0.29 dc	0.50bc	0.30b	0.79b
Helminthosporium	0.00b	0.00c	0.00b	0.00d	0.00c	0.00 d	0.37bc	0.21b	0.46b
Phylostica	0.00b	0.00c	0.00b	0.18d	0.12c	0.34 dc	0.00c	0.00b	0.00b
Clamydosporium	0.18b	0.12c	0.27b	0.37dc	0.22c	0.42 dc	0.47bc	0.29b	0.78b
Negrospora	0.38b	0.25c	0.41b	0.37dc	0.15c	0.42 dc	1.17bac	0.67ba	1.58ba

RD= Relative Density % IN= Infection rate % IF= Infection Frequency %
The original Data: square root transformed
Means with the same letter in a column are not significantly different

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