



RESEARCH ARTICLE

ASSESSMENT OF CHICKPEA SEED BORNE DISEASE WITH SPECIAL REFERENCE TO ASCOCHYTA
BLIGHT (*DIDYMELLA RABIEI*) IN CENTRAL ETHIOPIA

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ABSTRACT

Ascochyta blight caused by (teleomorph: *Didymella rabiei* (Kov.) is one of the most important seed borne pathogen of chickpea. The study was carried to determine the extent of seed borne infection. Agar plate techniques as ISTA standard method were applied. A total of one hundreds chickpea seed samples were collected from Debre Zeit, Gondar Agricultural Research Center, Farmers' seed and open market/union in 2015. Purposive sampling was used in areas affected by ascochyta blight. Hundred seeds were randomly taken from each sample. Seeds were surface disinfected in 0.5% (NaOCl) and rinsed three times in distilled water and seeds were transferred to chickpea meal extracts. Seed was scored and determine from fungal growth. The result indicates that the highest (18%) seed infection was recorded on one seed samples from research center, followed by (15%) and (14%) infection were recorded on seed samples from open market/union and farmers' seed and lowest (2%) infection of seed samples were recorded both on research centers and farmers seed. The seed harvested from all infected seed source contribute primary inoculums and long distance dissemination of spores. Seed health and free from seed borne diseases constantly desired to with reduction of seed borne inoculums through various seed treatments. In order to increase the production of chickpea qualitatively and quantitatively, farmers require healthy and quality seed with high germination and purity. Pathogen free healthy seeds are essential for desired plant populations and a good harvest.

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is widely grown crops over 14.8 million hectares of area globally with 14.24 million tons of grain legume is produced and play vital role in ensuring food and nutritional security in sub-Saharan country (FAOSTAT, 2014). The total area covered by chickpea in Ethiopia is estimated at 258,486.43 ha and from this a corresponding mean annual production of 472,611.4 tons of chickpea grain is produced (Central Statistical Agency, 2017). Globally, it is estimated that about 30% of plant diseases are seed-borne (Vishunavat, 2007). Although more than 15 fungal pathogens have been reported as seed borne pathogen on chickpea from different parts of world so far only a few of them are currently recognized as significantly important to chickpea production (Alemu and Sinclair, 1979; Pande *et al.*, 2010). Among the disease Ascochyta blight caused by *Ascochyta rabiei* (teleomorph: *Didymella rabiei* (Kov.) is one of the most important seed borne pathogen of chickpea (Singh *et al.*, 1984).

Seed borne pathogens have significant influence on seed production and food industry because they; (i) can affect germination, growth, and crop productivity, (ii) cause seed and seedling diseases resulting in the development of systemic or local infections, (iii) cause contamination of grains with mycotoxins that represent a health risk to humans and animals (Mukhtar, 2009; Singh *et al.*, 2011; Somda *et al.*, 2008). Damages such as seed death and decreased seed vigour caused by seed borne pathogens are not always recognized by users (Kakde and Chavan, 2011). Seed quality is very important in chickpea production since the cost of seed and potential seed treatments are a significant part of input costs (Penny, 2005). Ascochyta blight is a seed borne disease and infected seed is an important source of the primary inoculum in the field (Nene and Reddy, 1987) and epidemics can be initiated by very low levels of seed infection. Heavily infected seeds have discoloration and shriveled (Tivoli and Banniza, 2007). Seed and plant debris are main sources of the carryover of the fungus (Nene *et al.*, 1991). Planting infected seed allows for an even distribution of the disease within the crop and increases the number of initial infection sites from which the disease will spread during subsequent rain events. A good deal of research work has been done on the survival of the fungus through seed. (Luthra and Bedi, 1932) were probably the first to demonstrate

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the seed-borne nature of the pathogen (Halfon, 1970). confirmed the presence of the fungus in the seed coat and cotyledons, and of pycnidia in lesions. The seed coat and cotyledons of infected seeds contained mycelium and that the infected-seed weight was less than that of healthy-seed weight (Lukashevich, 1958a). showed that the fungus can behave as a saprophyte and spread to non infected tissues if the harvested material is stored for some time before threshing. He found 1.5 to 2-fold increases in seed infection during pre threshing storage (Maden *et al.*, 1975). Carried out a detailed study in Denmark on the seed samples received from Turkey. They found that 70 % of this seed from Central Anatolia was infected by *Ascochyta rabiei*. Pycnidia were observed only in the seed coat of seeds having deep lesions. Whole-mount preparations and microtome sections showed that inter and intracellular mycelium was localized in lesions. They established that both superficial and deep infections were equally potent in the transmission of the disease. All these studies considered together clearly establish the role of seed in perpetuating the fungus from one season to the next (Lukashevich, 1958a). Planting seed that is free of ascochyta blight and other pathogens is the primary means to limit the introduction of the pathogen into a field and prevent early establishment of disease (Penny, 2005). Recently, seed borne disease mainly ascochyta blight is increasing in its intensity and introduced even to new areas due to seed infection that suspected to spread from one site to another and hampered the quality of seed and transmission of pathogen seed to seedling at early seedling in Ethiopia. Seed borne pathogen *Ascochyta rabiei* where noticed as responsible for high gap yield potential. The objectives this study was to determine the extent of seed infection by ascochyta blight.

MATERIALS AND METHODS

Survey of seed infection of *Didymella rabiei* on chickpea

Collection of seed samples: Seed samples were collected from different farmers' fields of North and East Shewa Zones of major ascochyta blight disease affected areas, open markets and research centers. A total of hundreds chickpea seed samples of 0.5 kg seed per samples were collected. Purposive sampling method was used from selected woreda's. Thirty samples were collected from farmers saved seed of kabuli type of chickpea, thirty samples were from market and small retailer and forty samples were from Debre Zeit and Gondar research centers. Out of the forty samples fifteen samples were collected from North Gondar in West Balesa districts of Gondar Research sub-station (unpublished information and reports) and twenty five seed samples were from Debre Zeit Research Center) harvested in 2014 main cropping season from all testing site.

Determination of chickpea seed borne fungi

Agar Plate Techniques: ISTA 2008 techniques are a standard method for fungal isolation and identification was applied. Briefly, hundred seeds were randomly taken from each sample. Seeds were surface-disinfected by soaking in 0.5% sodium hypochlorite (NaOCl) solution for 10 min with constant agitation. Seeds were rinsed three times in sterile distilled water for a minute. Seeds were transferred to chickpea meal extract agar plate (chickpea seed meal 40 g, dextrose 20 g, agar 20 g). Seeds were placed on Petri dish to avoid cross-contamination, since its bigger seed 5 seeds per plate were

arranged. Seed were incubated 1-2 weeks at 20°C in dark near ultraviolet light used to encourage the development of fruiting bodies. Seed was scored for *Ascochyta rabiei* infection and to determine infected seeds from fungal growth emerge and confirmed conidia under microscope.

$$\text{The infection (\%)} = \frac{\text{Number of samples in which a disease infection occurred}}{\text{Total number of seed samples}} \times 100$$

RESULTS AND DISCUSSION

The present study results revealed that level of *Ascochyta blight* infection were significant from different sources of seed samples. The levels of infection were ranged from 2% to 18% for seed sources associated to seed borne fungi (Figure 1). The highest (18%) seed infection level was detected on one seed sample, that followed by (7%) seed infection on six seed samples and lowest (2%) were isolated on seven seed samples collected from Debre Zeit Agricultural Research Center. The seed borne pathogen invasion reduced germination, and nutritional and also responsible in producing mycotoxins and loss quality (Youssef, 2009). Dereje *et al.*, 2012 reported that seed-borne diseases serve as primary inocula for the infection of the next developing crops there by reducing yields and qualities of the produces and also play a role in spreading the diseases to new areas. In contrast, the highest (6%) seed infection were recorded on six seed samples and followed by (5%) were found on nine seed samples collected from Gondar Agricultural Research center. The large proportion of chickpea seed samples were collected from Debre Zeit Research Center were severely infected by ascochyta blight disease than seed samples from Gondar Research Center. The infection of *Ascochyta blight* were transmitted from pod infection to seed and responsible for yield reduction.

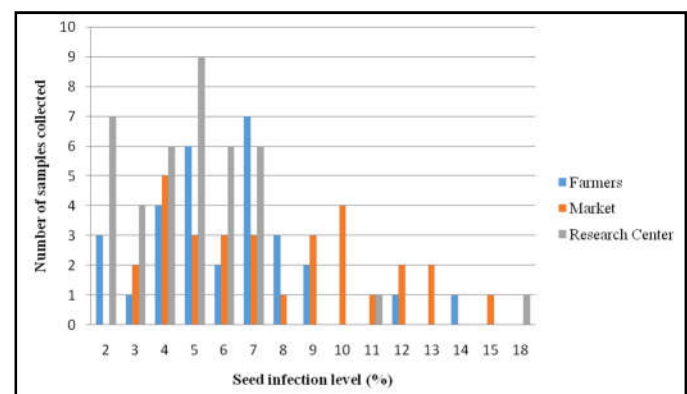


Figure 1. Evaluation of seed borne diseases with ascochyta blight infection levels from research centers, market and farmers seed lots in (2014)

The seed borne pathogens associated with seeds externally or internally may cause various infection like seed necrosis, reduction or elimination of germination capacity, as well as seedling damage resulting in development of disease at later stages of plant growth by systemic infection (Khanzada *et al.*, 2012). Infected seeds play a key role in the dissemination of plant pathogens and disease establishment (Raj *et al.*, 2007). Whereas, highest (14%) seed infection were recorded, followed by (12%) on two seed sample and lowest (2%) seed infection were recorded on three seed samples collected from farmer's stores (Figure 1). This implies that seed quality interms of seed borne disease inspection or seed health assessment not commonly applied with respective regional

seed enterprise and farmers cooperative union not strongly and follow rules of seed inspection and other informal seed produces so far. Within these seed flows or exchange on increment inoculum load of the pathogen year after year as seed multiplication in same fields. Several studies have been reported that the seed harvested from infected field by ascochyta blight disease serve as source of primary inoculum and long-distance dissemination of the spore (Singh *et al.*, 2011). In the majority of seed source, there were also low levels of contamination with other fungal pathogen such as *Aspergillus* spp, *Fusarium* spp, *Penicillium* spp from microscopic observation in seed sample. On other hand, the highest (15%) seed infection was recorded on one seed sample, (13%) infection of seed were recorded in two seed samples, followed by (12%) and (10%) infection and lowest (3%) infection were found on two seed samples. The seed infection level was higher on seed samples sources from market as compared to farmers' seed sources (Akema *et al.*, 2004). studied that seed borne pathogen has been implicated in the introduction of the disease into new areas or in the rapid spread of the disease within fields and seed treatment with effective fungicides can greatly help in reducing the initial inoculum level and preventing the spread of the disease. Seed lots collected from local market had more source of inoculum for disease because of seed collection and exchange from several locations and mixture compositions of desi with kabuli type of chickpea particular in union and other key actors in informal seed system. Infected seed play an important role in the epidemiology of the disease, ensuring random distribution of the pathogen can spread and produce secondary infection (Halfon, 1970). was the first scientist to report the infection of chickpea seed by *Ascochyta rabiei* and pathogens were transmitted from infected seed during germination. Seed treatments reduce seed infection by pathogen and improve germination capacity (Penny, 2005). Demonstrated that there is a high rate of seed-to seedling transmission of the pathogen, even a small amount of infected seed can result in significant seedling infection in the field. For example, with a 0.1 percent ascochyta-infected seed lot (one infected seed in 1000 seeds), potentially 175 infected seedlings per acre could result if planting density is 3 to 4 plants/m². Similarly, the disease infection was more on the seed lots having high percentage of infected seeds. Seed sample source even released from research center harbor more inoculum source of ascochyta blight pathogen which may seed harvested from ascochyta blight infected chickpea field or adjacent field to chickpea field and pathogen may not be seen on external surface of seed, internal in embryo, seed sample seems health with visual observation. The seed borne fungal diseases are transmitted by seeds, where the fungi can survive as conidia or mycelia on the seed coat or surface (Gargouri *et al.*, 2000). As a result, seed procured from blighted plants results in poor germination and show severe disease development (Kumar *et al.*, 1983). Sattar, 1933 reported that the surface contamination of seed with fungus spores and their role in causing infection. He found that 50% of such spores survived on seed for 5 months at 25-30°C. Further, it necessitates seed health and free from seed-borne diseases are constantly desired with the eradication of seed-borne inocula through various seed treatments.

Conclusion

Seed is the most important input for crop production. Production and productivity of chickpea seed were constrained seed borne pathogen. Epidemics of *Ascochyta* blight can be

initiated by very low levels of seed infections. The objectives of this study was to determine the extent of seed infection by *Ascochyta* blight of chickpea from open markets, chickpea producing farmers stores and research centers. Results from evaluation of seed infection level vary from different source of seed samples were ranged from 2% to 18%. The seed-borne pathogen may be present externally or internally or also associated with the seed as contaminant. Most of seed source not inspected before harvest and planting from different seed system. Legal inspection for certified seed not strong as set by seed regulation. Other gaps also seed infested by disease is negligence by seed producers and seed distributor unions. There was also little attention given to the transmission of pathogen from seed to plant. It is important to practices aimed at reducing distribution of seed borne pathogens for exchange of seed and collected materials. In order to increase the production of chickpea qualitatively and quantitatively, farmers require healthy and quality seeds with a high germination and purity. Seed treatment with effective fungicides can greatly help in reducing the initial inoculum level and preventing the spread of the disease. Hence, it is imperative that the seeds must be tested before sowing in the field. Seed health needs great concerns, especially to farmers and seed producers as well as seed certification schemes. Farmers should use certified seed only.

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