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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 for fungal isolation and identification were applied. A total of one hundreds chickpea seed samples were collected from Debre zeit and Gondar Agricultural Research Center, Farmers' seed and open market/union in 2014/15. Thirty seed samples from farmers, thirty seed samples collected from market and farmers' cooperative union and forty seed samples of 0.5 kg per samples were collected from both research centers. 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.

Key words: Ascochyta blight, Chickpea, *Didymella rabiei*, Pathogen, Seed borne disease

INTRODUCTION

Background and Justification: 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 (CSA, 2014). Seed borne pathogens have significant influence 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, Somda et al., 2008, Singh et al., 2011). 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). 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).

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 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.

METHODOLOGY

a) *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

Using ISTA (2008) techniques. 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 ^{o}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. The percentage of infection was calculated (Number of seed samples in which a disease infection occurred/total number of seed samples) x 100

RESULTS AND DISCUSSIONS

The present results of seed sample detecting of seed borne fungal infection were showed that significant difference on ascochyta blight infection levels from different sources of seed samples using the agar plate method on chickpea meal extract agar showed that the seed infection level ranged from 2% to 18% on seed samples from different sources which associated to seed borne fungi (Figure 1). The highest (18%) seed infection was recorded on one seed sample, (7%) seed infection were on six seed sample, (4%) infection were on six seed sample and followed by (3%) and (11%) infection of seed sample and lowest (2%) seed infection 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%) seed infection 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 seed borne pathogen transmitted from pod infection to seed and responsible for yield reduction. 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., 2002). Infected seeds play a key role in the dissemination of plant pathogens and disease establishment (Raj et al., 2007).

Whereas, the highest seed infection level (14%) were recorded, followed (12%) infection levels, (9%) seed infection were observed on two seed sample, (7%) infection were recorded on seven seed samples and lowest seed infection (2%) 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 inoculums 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. In the majority of seed source, there were also low levels of contamination with other fungal pathogen such as *Aspergillus* spp, *Fusarium* spp, *Pencilium* spp from

microscopic observation from seed sample plate on growth media.

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. Butler (1918) 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/m^2. 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.

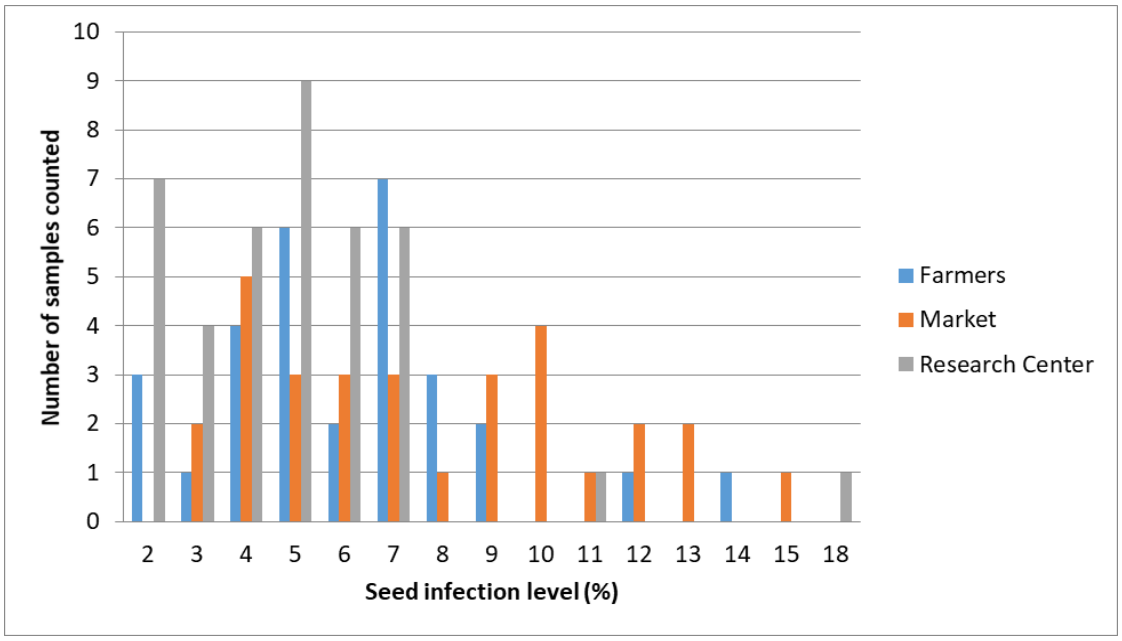

Fig 1. Evaluation of seed borne diseases with ascochyta blight infection levels from research centers, market and farmers seed lots in 2014 cropping season.

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 inoculums 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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REFERENCE

Akema, C., Kabbabeh, S., and Seid, A. (2004). Integrating cultivar resistance and seed with planting dates to manage chickpea Ascochyta blight. *Plant pathology Journal*, 2:111-117.

Alemu M and Sinclair JB (1979). Seed borne Microorganism grown Soybean and Chickpea seeds. *Ethiopian Journal of Agricultural Sciences*, 1:48-58.

Central Statistical Agency (CSA) (2014). Agricultural sample survey report on area and production of crops private peasant holdings, meher season. Pp 2-4. September–December 2014/2015 Volume I, Statistical Bulletin No. 388, Addis Ababa, Ethiopia.

Dereje G, Amare A, Frew M and Yonas N (2012). Seed health testing in seed systems in Ethiopia. 169-180. *In* Adefris, T., Asnake, F., Dawit, A., Lemma, D. and Abebe, K. (eds.) The Defining moments in Ethiopian Seed System. EIAR, Addis Ababa, Ethiopia.

FAOSTAT (2014). Availableonline:http://faostat.fao.org/site/567/DesktopDefault.aspx (accessed on 10 October 2016).

Gargouri S, Hajlaouri MR, Abdennadher M and Marrakchi M (2000). Isolation and morphological and molecular identification of Fusarium spp. transmitted by watermelon seeds. Bull OEPP/EPPO Bull 30:217-222 (in French).

Halfon MA (1970). Infection of chickpea seeds by *Ascochyta rabiei* in IstsebReporter.54: 442-445.

ISTA (2008). Handbook for Seedling Evaluation. International Seed Testing Association. Bassersdorf, Switzerland.

Kakde RB and Chavan AM (2011). Deteriorative changes in oilseeds due to storage fungi and efficacy of botanicals. Current Botany, 2: 17-22.

Khanzada KA, Rajput MA, Shah GS, Lodhi AM and Mehboob F (2002). Effect of seed dressing fungicides for the control of seed borne mycoflora of wheat. *Asian Journal of Plant Sciences* 4:441-444.

Kumar A, Jalali BL, Panwar MS and Sangwan MS (1983). Fungi associated with different categories of chickpea seed and their effects on seed germination and seedling infection. *International Chickpea NEWSLETTER*, 9: 19-21.

Lukashevich AI (1958a). Control measures against ascochytosis or chickpea (in Russian). *Journal of Agricultural Sciences* Moscow 5: 131-135 (*Rev. Appl. Mycol*. 37: 62. 1958).

Luthra JC and Bedi KS (1932). Some preliminary studies on pram blight with reference to its cause and mode of perennation. *Indian Journal of Agricultural Sciences* 2: 499-515.

Maden S, Singh D, Mathur SB and Nesrgaard P (1975). Detection and location of seed borne inoculum of Ascochyta rabiei and its transmission in chickpea. Seed Sciences and Technology 3: 667-681.

Mukhtar I (2009). Sunflower disease and insect pests in Pakistan: A review. *African Crop Science Journal*, 117: 109-118.

Nene YL and Reddy MV (1987). *In*: Chickpea Sexena, M.C., and Singh, K.B. (eds.) Wallingford, UK: CAB International. Chickpea disease and their control. Pp 233-270.

Nene YL, Reddy MV, Haware MP, Ghanekar AM and Amin KS (1991). Field Diagnosis of Chickpea Disease and their Control. Information bulletin no.28. Patancheru, Andhra, Pradesh, 502 324, India: International Crop Research Institute for the Sem-Arid Tropics, 29.

Pande S, Sharma M, Gaur PM and Gowda CLL (2010). Host Plant Resistance to Ascochyta Blight of Chickpea. Bulletin No 80: 1-8, India.

Penny P (2005). Seed quality in pulse crops. Saskatchewan Agriculture, Food and Rural Revitalization, Regina.

Raj MH, Narayan SR, Nayak SC and Shetty S (2007). Health status of farmer saved paddy, sorghum, sunflower and cowpea seed in Karnataka. *India world Journal Agricultural Sciences* 39:167-177.

Sattar A (1933). Occurrence, Perpetuation and control of gram (*Cicer arietinum* L.) blight caused by Ascochyta rabiei (pass) with special reference to Indian condition. *Journal of Applied Biology* 20: 612-632.

Singh KB, Reddy MV and Nene YL (1984). International testing of chickpeas for resistance to Ascochyta blight. *Plant disease*, 68: 782-784.

Singh S, Srivastava S, Shikha SA and Bose B (2011). Studies on seed mycoflora of wheat (*Triticum aestivum* L.) treated with potassium nitrate and its effect on germination during storage. *Research Journal of Seed Science*, 4: 148-156.

Somda I, Sanou J and Sanon P (2008). Seed-borne infection of farmer-saved maize seeds by pathogenic fungi and their transmission to seedlings. *Plant Pathology Journal* 7: 98-103, 2008.

Tivoli B and Banniza S (2007). Comparison of the epidemiology of Ascochyta blights on grain legumes. *European Journal of Plant Pathology* 119: 59–76.

Vishunavat K (2007). Seed health testing: Principles and protocols. Kalyani Publishers, New Delhi.

Youssef MS (2009). Natural occurrence of mycotoxins and mycotoxigenic fungi on Libyan corn with special reference to mycotoxin control. *Research Journal Toxins* 1: 8-22.