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Characterization and Identification of Chickpea Wilt/Root Rot Pathogens in North Shoa, Ethiopia

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Abstract

Chickpea wilt/root rot is the main biotic stress that reduces yields of chickpea in the major chickpea growing areas of Ethiopia. This study was carried out to isolate, characterize, and identify fungal pathogens that cause chickpea wilt/root rot and enrich the microbial culture collection of Ethiopian Biodiversity Gene Bank and make them accessible for further research.

Root samples were collected from infected chickpea host plants grown in three districts of North Shoa. The root samples were sterilized with two steps surface sterilization and inoculated on Potato Dextrose Agar. The fungal pathogens were isolated from the inoculated Potato Dextrose Agar after seven to ten days of incubation at 26±2 ^{O}C. The fungal pathogens were characterized using cultural characteristics and microscopic morphology techniques. Data comprising the symptoms of the infected host chickpea plants at the farm land, the cultural characteristics of the fungi on Potato Dextrose Agar, microscopic morphologies and standard fungal identification key were used to identify the fungal species.

The identified fungal pathogens include Fusarium oxysporum, Fusarium solani, Rhizoctonia bataticola, and Rhizoctonia solani. The rate of isolation of the pathogens is 20% for the three pathogens each; Fusarium oxysporum, Fusarium solani, Rhizoctonia bataticola and 10% to Rhizoctonia solani. Most of the pathogens are isolated from samples collected from Moretina Jiru district which indicates the existence of higher distribution of the disease in this district than the other two districts included in this study.

Keywords: *Wilt/root rot disease, characterization and identification of fungi, North Shoa*

Introduction

Chickpea (*Cicer arietinum* L.) is one of the legumes that are critical to smallholder livelihoods in Ethiopia. It contributes a lot to the economic, social and environmental benefits of Ethiopia (Mitiku, 2017). Ethiopia is the largest producer, consumer and exporter of chickpea in Africa and is also among the top ten most important producers in the world (Chichaybelu, 2021). It shares some 4.5% of global chickpea market and more than 60% of Africa's global chickpea market (Asrat, 2017; Damte and Ojiewo, 2016; CSA of Ethiopia, 2014/15).

Chickpea seed is recognized as a valuable source of dietary proteins (18 to 22%), carbohydrate (52 to 70%), fat (4 to 10%), minerals (calcium, phosphorus, iron) and vitamins. Its straw has also good forage value. In addition to its importance in human food and animal feed, chickpea plays an important role in improving soil fertility by fixing the atmospheric nitrogen requirement. It can fix up to 140 kg N per ha from air and meet most of its nitrogen requirement. In relation to the health aspects of including pulses in diets, as a micronutrient-rich food source, it helps to reduce inflammation in the gut, and has beneficial effect on serum cholesterol level, thus reducing cardiovascular disease risk (Mitiku, 2017).

Chickpea in Ethiopia is grown in *Woina Dega* (Midlands to highlands with altitudes between 1500m and 2600m above mean sea level.) agro-ecologies with a rainfall of 700mm-1300mm. It is mostly adapted to cool and moderate temperature regimes during the growing period (Asrat, 2017; Damte and Ojiewo, 2016). It is grown by over 1 million households on 13.2% of the total crop acreage and forms 14.8% of the total production in Ethiopia (Fikre, 2014). The largest growing regions are Oromia, (West, East, North West Shoa and Arsi zones), Amhara (South Gondar, North and South Wollo, North Shoa zones) and few districts of Tigray and SNNP (Southern Nations, Nationalities and People) regions (Asrar, 2017; Damte and Ojibwe, 2016). In the 2014/15 season,

the Amhara and Oromia regional states together accounted for 91.6, 94.9 and 96.3% households, total area and the total chickpea production, respectively (CSA of Ethiopia, 2015).

The production and the productivity of chickpea is constrained by several biotic and abiotic factors, Among the biotic constraints, fungal and viral diseases are the major yield limiting factors throughout chickpea producing countries in the world. The major fungal diseases of chickpea include Aschochyta blight (*Aschochyta rabiei*, Fusarium wilt (*Fusarium oxysporum* fs *ciceri*) and dry root rot (*Rhizoctonia bataticola*), while the wet root rot (*Rhizoctonia solani*) and collar rot (*Scerotium rolfsi*) are less important (Seid & Melkamu, 2006; Beniwal et al., 1992).

Chickpea production and productivity in Ethiopia has recently declined and high potential yield gaps because of several biotic and abiotic factors. The average chickpea yield in Ethiopia is usually below 2 t/ha although its potential yield is more than 5 t/ha (Asrat, 2017). This is resulted from susceptibility of landraces to terminal drought, heat and no protection against weeds, diseases and insect pests (Asfaw et al., 1994). One of the greatest biotic stresses reducing potential yields in Ethiopia is chickpea wilt/root rot caused by *Fusarium oxysporum* f.sp. *ciceris* which is serious problem especial in the rain fed area (Asrat, 2017). Root rot diseases that are caused mainly by *Rhizoctonia bataticola* (Dry root rot), *Fusarium solani* (Black root rot), and *Rhizoctonia solani* (Wet root rot) occur in the main chickpea growing areas of Ethiopia. But the major one is *Rhizoctonia bataticola* (Dry root rot) [Mitiku, 2017]. When conditions are suitable for disease development it can cause yield loss up to 50%. Asrat (2017) indicated in his review that wilt/root rot caused yield loss of 50-80% in some farmers' chickpea fields and sometimes even 100% loss on local variety in North Western and Central Ethiopia.

A field survey study done by Damte and Ojiewo (2016) in some Shoa and Gojam zones during

2013-2015 revealed that wilt/root rot disease of chickpea is prevalent in all of the chickpea fields. As indicated in their study, the maximum disease incidence was recorded in *Zjenji* area where the entire field was wiped out by the disease. Yimer et al. (2018) recently reported, based on the results of their survey of five major chickpea growing regions covering 30 districts in the central and northern highlands of Ethiopia, that the major pathogens associated with infected roots are *Fusarium oxysporum* f.sp. *ciceris*, *Fusarium solani*, *Rhizoctonia bataticola*, *Sclerotium rolfsii*, and *Rhizoctonia solani*. The most frequently isolated pathogen was *F. oxysporum* f.sp. *ciceris* followed by *R. solani*.

This study was done to isolate, characterize, and identify fungal pathogens that cause chickpea wilt/root rot in North Shoa district. And to use the fungal pathogens as test strains for further biological control research. In addition, the study was carried out with the intention of enriching the microbial genetic resources of Ethiopian Biodiversity Institute's gene bank.

Materials and Methods

Sample collection

Samples were collected from major chickpea growing districts of North Shoa Zone where Wilt/Root Rot disease is prevalent. Information about the chickpea growing districts were gathered from the zonal agricultural bureau and those districts which are severely affected by wilt/root rot diseases were selected for collecting the samples. The chickpea root samples were collected from infected chickpea crop in the three districts, namely; *Basona Worana, Ensaro*, and *Moretina Jiru*. Moreover, data on the cropping system, soil types, altitudes, latitudes, longitudes, and growth stages of the chickpea crops were registered. The GPS data recorded at the time of sample collection shows that the sampling areas are located between latitudes of 09^{O}44.1860' N –

09^{O}59.2960' N, longitude range of 038^{O} 54.6593' E – 039^{O} 27.2264' E, and altitude range of 2505m - 2652m a.s.l.

Field diagnosis of wilt and/or root rot complex diseases

Samples were collected from 10 infected host chickpea plants showing the symptoms of WRR disease from 3 severely affected chickpea farms (Teixeira et al., 2016). Three to four spots were taken in one chickpea farm field to collect diseased chickpea roots. Infected chickpea plants with any or combinations of the following symptoms of fungal diseases affecting root/stem base were selected from the infested chickpea farms (Mitiku, 2017; Nene et al., 2012; Nene et al., 1978):

Fusarium wilts [*Fusarium oxysporum* f. sp. *ciceri*]

Symptoms: The field symptoms of wilt are dead seedlings or adult plants, usually in patches. The disease can affect the crop at any stage. It causes wilting, yellowing, vascular discoloration and death of chickpea plants (Westerlund et al, 1974).

Seedling stage: Whole seedlings (3 - 5 weeks after sowing) collapse and lie flat on the ground. These seedlings retain their dull green color. When uprooted, they usually show uneven shrinking of the stem above and below the collar region (soil level). The shrunken portion may be about 2.5 cm or longer. Affected seedlings do not rot on the stem or root surface.

Adult stage: The affected plants show typical wilting, i.e., drooping of the petioles, rachis and leaflets. The leaves are yellow and light brown or straw colored. Dried leaflets of infected plants are not shed at maturity. Affected plants, when uprooted and examined they show no external rotting, drying, or root discoloration.

Wet root rot [*Rhizoctonia solani*]

Symptoms: The field symptoms are drying plants scattered throughout the field. This disease is most often seen at the seedling stage (up to 6 weeks after sowing) in soils with relatively high

moisture content. Affected seedlings are yellow, and petioles and leaflets become drooped but not collapsed. A distinct dark brown lesion appears above the collar region on the main stem of older plants and their stem and root below the lesion show rotting, frequently with pinkish mycelial growth.

Dry root rot [*Rhizoctonia bataticola*]

Symptoms: The disease generally appears around flowering and podding time in the form of scattered dried plants. Drooping of petioles and leaflets is confined to those at the very top of the plant. The leaves and stems of affected plants are usually straw colored, but in some cases the lower leaves and stems are brown. The lower portion of the tap root usually remains in the soil when plants are uprooted. The tap root is dark, shows signs of rotting, and is devoid of most of its lateral and finer roots.

Black root rot [*Fusarium solani*]

Symptoms: The disease is seen at any stage of the plant. Affected plants turned yellow and wilt. Dead plants are seen scattered in the field. The root system is rotten, most of the finer roots are shed, and the remaining roots turned black. Affected plants dried prematurely.

Isolation and purification methods of fungal pathogens

The affected root of the plant tissues was washed thoroughly in sterile water. Portion of plant tissue exhibiting clear symptoms was cut along with adjacent small unaffected tissue into small pieces (2-5 mm squares). Two steps surface sterilization was used to sterilize the plant tissue (Narayanasamy, 2011). The plant tissues were surface sterilized by transferring them into sterile Petri-dishes containing Sodium hypochlorite (1%) solution using sterilized forceps for a period of 2 minutes and rinsed it with sterile water. Then, the tissues were transferred into 70% ethanol for

1 minute and rinsed twice with water. Aseptically, the sterilized plant tissue pieces were transferred to Potato Dextrose Agar (PDA) supplemented with Chloramphenicol (300g/l), at the rate of three to five pieces of tissues per Petri-plate and incubated at 26^{O}C for seven to ten days (Al-Fadhal, 2019). All the plates were grown in triplicates for the complete isolation and purification of plant pathogenic fungi (Thilagam et al., 2018). A portion of mycelium developing on the PDA was transferred to the PDA for purification and the purified fungal disks were stored in Potato Dextrose Broth with 10% glycerol at 4 ^{O}C for further examination (Narayanasamy, 2011).

Characterization methods of fungal pathogens

The cultures grown on PDA were used to study the cultural characteristics and morphology of the fungal pathogens. After seven to ten days of incubation, colony diameter, sporulation, colony base and surface character, and pigmentation were recorded by observing through a magnifying glass lens. The cellular structures of the isolated fungi were examined to characterize their microscopic morphology using light compound microscope after staining the specimens with lacto-phenol cotton blue (Ploetz and Freeman, 2009; Leslie and Summerell, 2006; Watanabe, 2002; Woodward, 2001; Barnett & Hunter, 1998).

Identification methods of fungal pathogens

Standard fungal identification key was used to differentiate the genera/species (Watanabe, 2002; Ploetz and Freeman, 2009; Leslie and Summerell, 2006; Woodward, 2001; Barnett & Hunter, 1998). The cultural and morphological characteristics of the isolated fungal species were additionally triangulated with other literatures (Gaikwad et al, 2020; Al-Fadhal et al, 2019; Teixeira et al, 2016).

Results

Isolation of fungal pathogens

Seven fungal pathogens were isolated from the collected chickpea roots on Potato Dextrose Agar (PDA) supplemented with Chloramphenicol (300g/l) at incubation temperature of 26 ^{O}C for seven to ten days. The isolated fungal pathogens are shown in table 1 with the *woreda* (District) name from which the sample materials were obtained. The isolated fungi were labeled as CPDR5A, CPDR5C, CPDR6B, CPDR7A, CPDR7B, CPDR8A, and CPDR9A. Five of the seven fungal pathogens isolated were from the infected chickpea roots collected from farms that are found in *Moretina Jiru woreda* (District), and two of them are isolated from samples collected from *Ensaro* districts, respectively.

Table 1: List of the isolated chickpea fungal isolates and their locations

S. No.	Isolated Fungi	Locations/Districts
1	CPDR5A	*Moretina Jiru*
2	CPDR5C	*Moretina Jiru*
3	CPDR6B	*Moretina Jiru*
4	CPDR7A	*Moretina Jiru*
5	CPDR7B	*Moretina Jiru*
6	CPDR8A	*Ensaro*
7	CPDR9A	*Ensaro*

CPDR: Chickpea-diseased root

Characterization of the isolated fungi

The isolated chickpea wilt/root rot fungal pathogens were characterized by using cultural and microscopic morphology characterization techniques.

Cultural characterization

Four peculiar colonies of fungal pathogen were detected among the seven fungal isolates of the infected chickpea plants. These are indicated in fig. 1 and table 2.

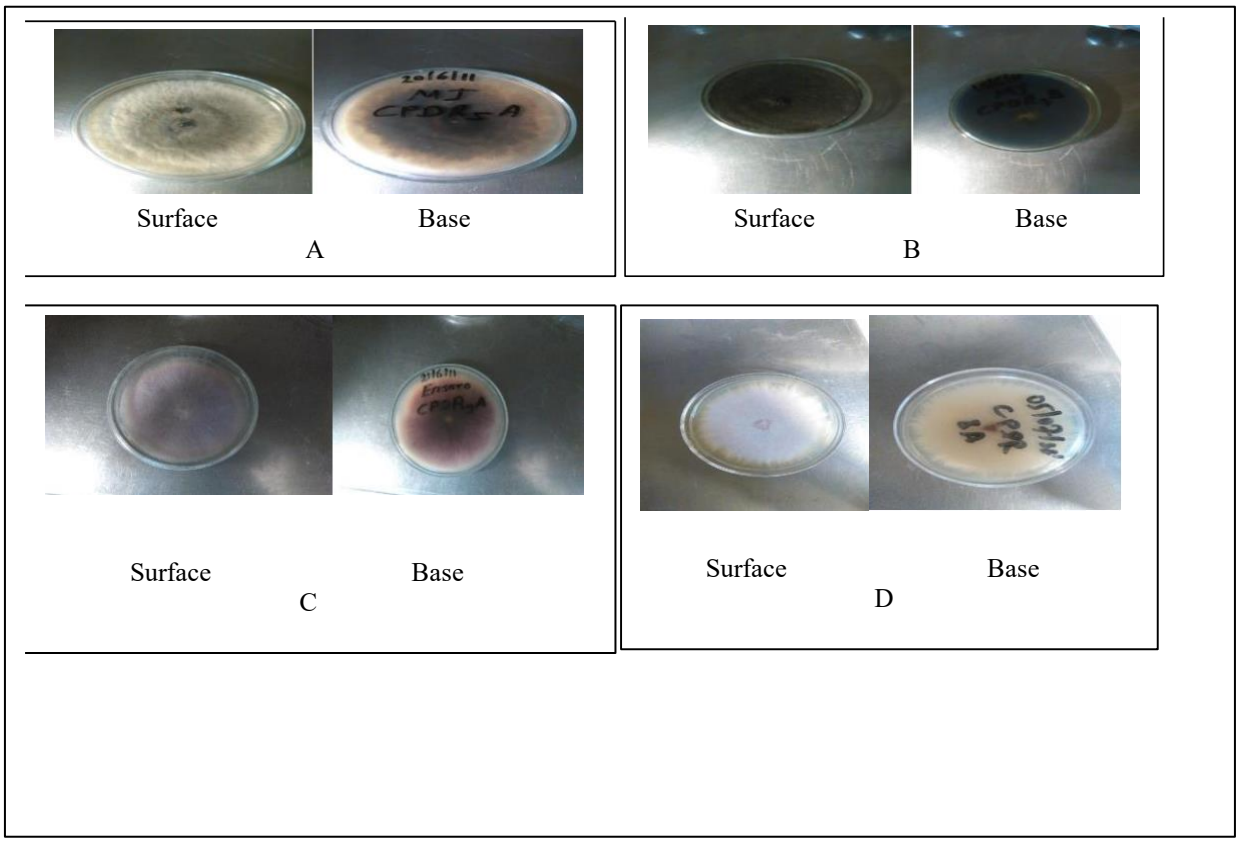

Fig. 1: Representative chickpea wilt/root rot fungal pathogens grown on PDA;

A: *Rhizoctonia solani*, B: *Rhizoctonia bataticola*, C: *Fusarium oxysporum* and D: *Fusarium solani.*

The description of the colony morphologies of the fungal pathogens that were isolated from infected chickpea having wilt/root rot symptoms are specifically shown in table 2. The morphological characteristics of the fungal isolates labeled CPDR7a and CPDR7b (*Rhizoctonia bataticola*) on PDA were black colony base and light black surface color, and the surfaces showed no or little arial mycelia and no spore. The colony size ranges from 8.5 to 9 cm diameter. The colony of fungal isolate labeled CPDR5a (*Rhizoctonia solani*) have pale brown base and light grey

surface which have 9 cm diameter. The surface has no arial mycelia and spore. Fungal isolates: - CPDR 6b and CPDR 9a (*Fusarium oxysporum*) have colony characteristics of moderate fluffy mycelium surface with white spores and pink basal mycelium. Their colony size ranges from 7.5 cm to 7.8 cm on the seventh day of incubation. Fungal isolates: - CPDR5c and CPDR 8a (*Fusarium solani*) have hairy white mycelia with white spore on light pink surface and light-yellow base. Their colony size ranges from 8.0 cm to 8.6 cm on the seventh day of incubation (Table 2).

Table 2: Cultural characteristics of the isolated chickpea WRR fungal pathogens

S.No.	**Isolate ID**	**Cultural characteristics**		
		Colony diameter	Sporulation	Colony characteristics and
1	CPDR5a	9 cm	No spore	Flat hairy white grey mycelia, pale
2	CPDR5c	7.5 cm	White spore	Hairy white mycelia at surface and
3	CPDR6b	8 cm	White spore	White cottony mycelium surface, pink
4	CPDR7a	8.5 cm	No spore	Black surface and base with no aerial mycelium
5	CPDR7b	8.5 cm	No spore	Black mycelia, white hairy mycelium at the colony periphery
6	CPDR8a	7.8 cm	White spore	Moderate fluffy white mycelium, light
7	CPDR9a	8.6 cm	White spore	Moderate fluffy white mycelium, pink

Microscopic morphology characterization

The microscopic morphologies of the isolated fungal pathogens were characterized and primarily used to identify the species of the causal agents (Table 3). The microscopic morphologies of fungal isolates: - CPDR 6b and CPDR 9a (*Fusarium oxysporum*) showed features which are lunar or banana shaped conidia (microconidia, macroconidia), chlamydospores and conidiophores shorter than macroconidium width. The microscopic morphologies of fungal isolates: - CPDR5c and CPDR 8a (*Fusarium solani*) showed banana shaped conidia (microconidia, macroconidia) and conidiophore longer than macroconidium length by few times.

Table 3. Microscopic morphology characterization results of Chickpea WRR fungal pathogens

S.No.	Isolate ID	Microscopic morphology	
		Hyphae	**Conidia, spore and conidiophore**
1	CPDR5a	Septate near/at branching point (right angled branches)	No conidia, dark sclerotia, monilioid cells
2	CPDR5c	Tiny hyaline, aseptate hyphae	Conidiophore longer than macroconidia (simple and branching), slightly curved brightly colored 3-4 elongated macroconidia
3	CPDR6b	Aseptate, non-branching	Brightly colored, 4 celled macroconidia, twins chlamydospores, conidiophore shorter than macroconidia
4	CPDR7a	Septate near/at branching point (right angled branches)	No conidia, dark brown sclerotia
5	CPDR7b	Septate at branching point (right angled branches)	No conidia, discrete sclerotia, various in shape: Dark brown circular/round sclerotia
6	CPDR8a	Aseptate, hyaline hyphae,	Simple and short branched conidiophore, conidiophore longer than macroconidia, Conidia/ spores hyaline, typically two celled ovoid conidia

7	CPDR9a	Aseptate, hyaline hyphae	Blue pigmented conidia, colorless branching conidiophores shorter than macroconidia width, three-4 celled curved macroconidia, Chlamydospore, brown phialide

The microscopic morphology of the fungal isolates labeled CPDR7a and CPDR7b (*Rhizoctonia bataticola*) were observed under microscope as septate mycelium and dark brown in colour. Typical right-angled branching of mycelium was observed. Sclerotia were discrete and various in shape including dark brown circular/round sclerotia to irregular. The fungal isolate labeled CPDR5a (*Rhizoctonia solani*) was seen under microscope as a septate mycelium and dark brown in colour. Typical right-angled branching of mycelium was observed. Sclerotia were dark brown and there were monilioid cells.

Identification of the isolated fungi

The fungal isolates were identified to the species level by combining the data gathered from the three characterization criteria. The symptoms exhibited by the infected host chickpea plants at the farm land, the cultural characteristics of the isolated fungi on PDA incubated at 26 ^{O}C from seven to ten days, and microscopic morphologies that were observed under light compound microscope were combined together. In addition, the results were triangulated with other standard manual and literatures written in relation to chickpea WRR fungal pathogens. Based on these criteria, seven of the isolated fungal pathogens were identified into four fungal species (Table 4).

Table 4: Key cultural characteristics and morphology used to identify WRR fungal Species

S.No.	Isolate(s) ID	Cultural characteristics	Micromorphology	Fungal Species
1	CPDR5a	Pale brown base and light grey surface, no spore	No conidia, Septate near/at right angled branching, dark sclerotia, monilioid cells	*Rhizoctonia solani*
2	CPDR5c And CPDR8a	Hairy white mycelia with white spore on light pink surface and light-yellow base	Conidiophore longer than curved macroconidia	*Fusarium solani*
3	CPDR6b and CPDR9a	Fluffy mycelium surface with white spores and pink basal mycelium	Conidiophore shorter than curved macroconidia	*Fusarium oxysporum*
4	CPDR7a and CPDR7b	Black colony base and light black surface color, no spore	No conidia, Septate near/at right angled branching, dark brown sclerotia	*Rhizoctonia bataticola*

Discussion

Most of the pathogens are isolated and identified from the samples collected from *Moretina Jiru* district. There is also multiple infection of chickpea root caused by Rhizoctonia *solani* and *Fusarium solani* in one of the host plants that were sampled from *Moretina Jiru* (Table 1 and Table 2). The rest two pathogens are isolated from samples collected from *Ensaro* district. This might be taken as an indication of more prevalence of chickpea WRR diseases in *Moretina Jiru* than the other two districts. The co-infection case shows the trending belief that the causative agents of wilt/root rot diseases of chickpea occur together (Damte & Ojiewo, 2016).

The seven fungal isolates of Chickpea WRR were identified into two genera and four fungal pathogenic species. Four of the seven isolates are under Fusarium genera and the rest three isolates are under Rhizoctonia genera. These are: *Rhizoctonia solani*, *Rhizoctonia bataticola*, *Fusarium oxysporum* and *Fusarium solani* (Fig. 1, Tables 3 and 4). These fungal pathogens are well known in causing chickpea WRR diseases in Ethiopia (Mitiku, 2017; Damte & Ojiewo, 2016; van Rheenen et al., 2011). The frequency of isolation of these WRR fungal pathogens from the host plants are 20% (2/10) for *F. solani*, *F. oxysporum*, *R. bataticola* each and 10% (1/10) for *R. solani*, respectively. Generally, the frequency of isolation of Fusarium pathogenic species (40%) from the collected samples is greater than that of the Rhizoctonia pathogenic species (30%) which cause chickpea WRR complex diseases.

The characteristics that are used to differentiate the species of the four isolates under Fusarium genera is that *Fusarium oxysporum* has conidiophore shorter than the curved macroconidium width but *Fusarium solani* possess conidiophore longer than the curved macroconidium length by few times (Watanabe, 2002; Ploetz and Freeman, 2009; Woodward, 2001; Barnett & Hunter, 1998). Concerning the Rhizoctonia species, the cultural and morphological characteristics of mycelium and sclerotia were in agreement with the descriptions stated in identification books (Desvani, 2018; Kuirya, 2014; Watanabe, 2002; Woodward, 2001; Barnett & Hunter, 1998). Thus, the three fungus isolates under the rhizoctonia genera were identified as two Rhizoctonia species. The two Rhizoctonia species are *R. bataticola* and *R. solani.* They are differentiated into species level by their own peculiar disease and cultural characteristics and presence of septate near/at right angled branching, dark sclerotia, and monilioid cells. The presence of monilioid cells in *Rhizoctonia solani* differentiate it from *Rhizoctonia bataticola.*

Conclusion

In this study, fungal pathogens that cause chickpea wilt/root rot are characterized and identified from samples collected from North Shoa districts using cultural characteristics and microscopic morphology. These fungal pathogens include *Fusarium oxysporum, Fusarium solani, Rhizoctonia bataticola, and Rhizoctonia solani.* The rate of isolation of the pathogens is 20% for the three pathogens *each; Fusarium oxysporum, Fusarium solani, Rhizoctonia bataticola and* 10% to *Rhizoctonia solani.* Most of the pathogens are isolated from samples collected from *Moretina Jiru* district which indicates the existence of higher distribution of the disease in this district than the other two districts included in the study.

Declaration of conflict of interest:

The authors declared that they have no conflict of interest.

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