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Research Article

Assessment of Root Rot Pathogens of Common Bean (*Phaseolus Vulgaris* L.) and Reaction of Genotypes to the Pathogens in West Hararghe Zone, Ethiopia

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Abstract

Common bean root rot caused by fungal pathogens is an important disease affecting common bean crops in Ethiopia. Information on pathogen identification, characterization, and management options is lacking for the Ethiopian bean production system. This study aimed to assess the major causal fungal pathogens and their management through host resistance methods. Initially, a field survey was conducted in three districts in 2016. It was cored forty-five (45) common bean fields. In the mean time, disease samples were collected for laboratory analyses. Secondly, pathogen identification and characterization were done in Laboratory at Haramaya University, followed by a pathogenicity test. Thirdly, a genotypic reaction was done on twenty common bean varieties by using four fungal genera (F. oxysporum, S. rolfsii, M. phaseolina, and R. solani) as experimental materials that were arranged in (CRD) design with three replications. Out of forty-five (45) common bean fields assessed 33 farms exhibited the disease. In the pathogenicity test, all the isolates were found pathogenic and showed a significant ($p < 0.05$) difference. In addition, the analysis of variance also showed that out of the tested twenty varieties, some released varieties (Dandesu, Tinike, SER-125, Dursitu, and Chorie), Chorie and (Dursitu, Chorie, Cranscope, Argene and SAB 632) showed highly significant at ($p \leq 0.001$) to Fusarium oxysporum f.sp. phaseoli, Sclerotium rolfsii, and Rhizoctonia solani while they didn't exhibit any significant ($p < 0.05$) difference to Macrophomina phaseolina. In conclusion, those varieties showing resistance characters were recommended for growers.

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most widely distributed species of the genus *Phaseolus* as it is grown in all the words with a broad range of adaptations to various environmental conditions [1]. It is one of the most important legumes because of its high commercial value, extensive production, consumer use, and nutrient value [2].

The economic significance of the common bean in Ethiopia is quite considerable since it represents one of the major food and cash crops. It has great potential for the country as it has been duly recognized by many researchers and organizations for its economic importance and its domestic demands for various uses. Production of this crop is indispensable in the country to enrich the staple cereal crops with sufficient and high-quality protein to overcome the problem of malnutrition, leading to marasmus or kwashiorkor [3]. Under optimal management conditions, the productivity of the common bean can reach up to 2.5 to 3.0 tons per hectare in Ethiopia [4]. However, the actual average production from 2008 to 2010 was only 1.4 tons per hectare due to different factors [5], which is very far below the crop potential yield.

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The major production constraints of common bean include moisture stress, diseases, insect pests, weeds, poor soil fertility, and lack of improved seeds [6], of which diseases are known to be the major factors that threaten the productivity of common bean in all growing areas [7]. Among many diseases affecting common beans, root rot (*Fusarium oxysporum*), and common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli* and halo blight caused by *Pseudomonas syringae* pv. *phaseolicola,* are the most destructive diseases of beans worldwide [8]. On the contrary, Tadesse, T. *et al*. [3] reported that the geographical distribution of root rot complex on common beans in Ethiopia was unknown and consequently considered as minor importance. Some informal reports from West Hararghe Agricultural Office and Mechara Agricultural Research Center indicated the problem with common bean production in the Zone, where the importance of the disease is required to be verified under a formal survey program. Experiences and evidence from other areas indicate that common bean root rot complex can cause significant damage and yield reduction depending on favorable conditions for the occurrence and development of the disease. The pathogens associated with root rot complex disease have wide host ranges, such as cereals, including wheat, sorghum, and legumes [9]. These soil-borne fungi include *Fusarium oxysporum* f.sp. *phaseoli*, *Rhizoctonia solani* and it may cause 50% yield losses in susceptible varieties [10,11]. In some countries, like Iran, estimates of yield losses range between 3.8% and 76.0% depending on the incidence of root rot disease and fewer seeds per plant under commercial production conditions [12]. In the Lake Victoria Basin and Mbale Farmlands, agroecology presented the highest (about 38%) incidences of Fusarium root rot. In Kanungu in the South Western Highlands, the incidence of Fusarium root rot was 23% [10].

As noted by Otysula *et al.* (2003), western Kenya experienced bean yield loss of more than 70% due to *Pythium* root rot under favorable conditions when susceptible varieties are used, leading to price fluctuation and low marketability. In Uganda, the incidence of these four pathogens may cause losses of up to 100% in susceptible varieties under moist conditions and impoverished soils [10]. The disease is characterized by aboveground symptoms, such as poor seedling establishment, uneven growth, and premature defoliation of severely infected plants [13]. Infected tissues become spongy, wet, and discolored with many cavities. In addition to the previous symptoms, the disease is also characterized by lower leaf yellowing (similar to nitrogen deficiency), stunting, leaf browning, and plant death [14].

Considering the nature of the damage and the survival ability of the pathogen, the use of resistant varieties is the only economical and practical solution. As a result, certified seeds of tolerant or resistant varieties to *Fusarium oxysporum* f.sp. *phaseoli*, *Sclerotium rolfsii*, *Macrophomina phaseolina,* and *Rhizoctonia solani* root rots are recommended for bean growers since this approach can be the best way for increasing production [15]. In Ethiopia, common bean root rots are widely distributed in every part of the country, especially the diseases highly occur in bean-growing areas of eastern Oromia [16]. The diseases frequently occurred in West Hararghe Zone and are assumed to cause great threats to the areas. But it has been considered too complex to identify the causal agents as well as to recommend viable disease management options. Therefore, studies towards assessment of the disease distribution, identification of the causative agent(s), and evaluation of alternative disease management strategies are highly important. Furthermore, the common bean breeding program has mainly targeted aerial and foliar diseases, whereas breeding for resistance against root rot complex has not been systematically conducted for common bean genotypes. Hence, information gaps in this respect have been addressed for the major soil-borne root rot complex disease, the aggressiveness of associated pathogens, and reactions of released genotypes against the root rot pathogens.

Characterization and identification of bean root rot complex-causing microorganisms as well as screening resistant bean cultivars were considered as the best solution to overcome the common bean root rot complex constraints. As a result, screening the improved and resistant/tolerant released common bean varieties against root rot complex in the target areas would be of paramount importance to increase common bean production and productivity. The study has addressed such questions as: What were the major organisms that caused root rot complexes? Which pathogen was the most aggressive of all collected and associated/identified pathogens? Which of the selected common bean genotypes was resistant/tolerant or susceptible to the aggressive pathogen? Answers based on practical and observed studies under laboratory, greenhouse, and field surveys to the above questions would help to develop management strategies against the root rot diseases and to minimize yield lose due to the diseases. Therefore, this study was designed with the following specific objectives:

To assess and determine the incidence and prevalence of root rot pathogens of common beans in West Hararghe,

To identify the major common bean root rot-causing fungal species and the aggressive nature of the pathogenic fungi associated with the disease on common bean; and

To evaluate the reaction of selected common bean genotypes against the most aggressive pathogens under glasshouse conditions.

Materials and methods

Description of the survey areas

A field survey was conducted to assess the incidence and prevalence of root rot complex of common beans in three districts of West Hararghe Zone during the 2016 main cropping season. Figure 1 represents the map of the West Hararghe zone showing the places where the samples were collected during our survey. The districts were purposively selected to represent the major common bean-growing areas of the Zone. Sites and farmers' field selection was carried out through discussion with the development agents (DA) and experts of the respective districts along with evaluation of secondary data. Group discussions with growers were conducted with the objective

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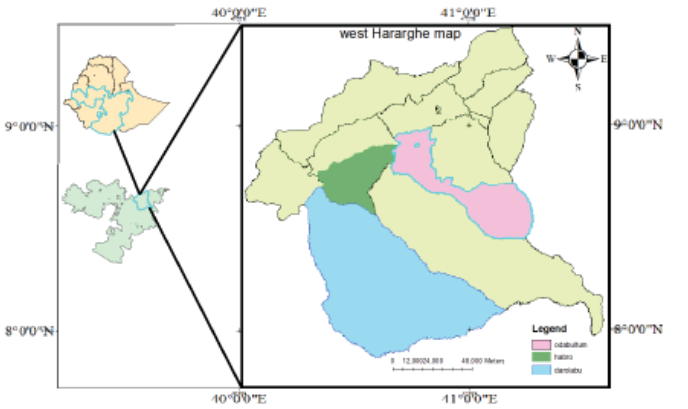

Figure 1: Map showing common bean root rot survey districts of West Hararghe Zone during the 2016 main cropping season.
Source: Ethio-GIS Lab in Haramaya University.

to extract information regarding constraints in common bean production, especially the incidence of common bean root rot complex diseases and their distribution, damage, area coverage, and their traditional management measures. Semi-structured questionnaires were developed for the purpose. Similarly, field inspection formats were developed to record data related to farmers' agronomic practices like-planting methods (sole and intercropping), planting time (March-April, May-June, and June-July), seed source (farmers' saved, market, Research Center, Union), pesticide usage and crop rotation system during field visits. Interviewing and observing a total of 45 farmers from representative common bean growers FAs and their field status of current cultural practices in the selected districts as stated in the Questionnaires (Appendix Table 6).

During the survey, a total of 45 farmers' fields were observed and three to five plant samples from each field were collected. This was done based on different naturally infected plant parts (roots and stems), which showed the suspected typical symptoms of root rot complex. Ecological data and background information of each sample were recorded, separately maintained in paper bags, and taken to the laboratory for further analytical work.

Sampling procedures

From each of the three surveyed districts, three common bean-growing Farmers' Associations (FAs) were purposively identified for the disease survey. From each of the Farmers' Associations, five common bean fields separated by a minimum of 4-5 km distance were considered in the disease survey and assessed for incidence and distribution of root rot complex. A random sampling of the fields for disease assessment was carried out by moving in a 'W' fashion within an area of a half hectare 0.5 ha in the field by taking plants at approximately 5 m intervals. The incidence of root rot was recorded from each sample quadrat of 1 m*1 m. The incidence of the disease in the whole field was then determined as the percent of diseased plants out of the five assessed quadrats.

Disease incidence and prevalence assessment

Common bean root rot incidence was assessed by counting the number of plants showing the disease symptoms in each quadrat and expressing as a percentage of the total number of plants assessed, while disease prevalence was assessed using the number of fields affected divided by the total number of fields assessed per district and expressed in percentage [17]. The following formulae were used to calculate the percentage of disease incidence and disease prevalence:

$$\text{Disease incidence}(\%) = \frac{\text{No.of infected plants}}{\text{Total no.of plants assessed}} \times 100$$

$$\text{Disease prevalence}(\%) = \frac{\text{Fields with disease symptoms}}{\text{Total fields assessed}} \times 100$$

Samples of diseased plants were collected from the sampled common bean fields and were transported to the Plant Pathology Laboratory of the School of Plant Sciences, Haramaya University, for isolation and identification of major root rot pathogens as described in the Section below.

Data collected

Other common bean diseases were also recorded in each assessed field. The following data on-site, crop, and agronomic parameters were also collected during the survey. Crop variety: Common bean variety used was obtained from the growers through interviews. Crop growth stage: Direct record was made on the stage of the crop growth stages (seedling, vegetative, flowering, podding, and physiological maturity stage) during the survey. Soil type: Data on the soil type where the crop was grown was recorded as sandy, silt loam; clay-loam, loam, and others by visual observations, MARC soil result data, and woreda soil profile. Planting date: The planting dates of the common bean crops were obtained from the growers through interviews (March-April, May–June, and/or July-August). Cropping system: The types of cropping systems (sole cropping, row intercropping, mixed intercropping, etc.) used by the farmers were recorded by direct field observation. Plant density: It was determined by counting the number of plants per quadrat of each field. Altitude: The elevations of the fields had been recorded by using Global Positioning System (GPS) in the three districts during the survey. Meteorological data: Rainfall and temperature data of the three districts were obtained from the meteorological station of Mechara Agricultural Research Center, Habro meteorological station, and the National meteorological agency for Oda Bultum district. Researchers also used rainfall status based on its continuation and grouped as (before 15 days considered as dry, a week considered as moderate moisture, 2-3 times within three days considered as adequate moisture) in the surveyed areas.

Data analysis for disease survey

The mean incidence and prevalence of root rot were calculated for each district by descriptive statistics (Gomez and Gomez, 1984).

Activity 2. Identification and Characterization of Common Bean Root Rot Pathogen(s)

Sample collection

During the surveys, plant samples showing typical symptoms of root rot were collected from each common bean

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field. Symptoms such as yellowing, wilting, and stunting were considered as guides for sample collection. During sampling, quadrats were used, and counting plants in it manually and the results were jotted down on the survey data record sheet by categorizing the diseased and normal by adding them to get the total plant in the quadrat. These finally were grouped as 10-30 considered as minimum plant density, 31-60 considered as optimum density and > 60 high plant density for the purpose of data management. From each field, 3-5 symptomatic sample plants were collected at different points of the field. Plants had been uprooted and shaken to remove adhering soil. Specimens were packed in paper bags, labeled, and then transported to the Plant Pathology Laboratory of the School of Plant Sciences, Haramaya University, for isolation and identification of the causal organisms. The samples were stored in the refrigerator at 4 °C until fungal isolation was performed.

Isolation and identification of root rot pathogens

A sample having suspected disease symptoms were uprooted and washed with a jet stream of tap water to remove the soil from the plant tissues, rinsed twice in sterile distilled water, blotted dry on a new paper towel, then dissected infected root pieces (approximately 0.5 to 2 cm long) cut from the edge of the diseased part and surface-sterilized in 10% sodium hypochlorite solution for 3 min and rinsed 5 times by changing sterile water. The sterilized pieces were put on a potato dextrose agar (PDA) medium for the isolation of fungal pathogens. Petri plates with plant samples were incubated at 25 -27 ºC and were observed 24 and 48 hr after incubation. After a few days of growth, each type of pathogen was purified by transferring cultures to a fresh PDA medium and then streaking a diluted hyphae suspension on a transparent agar. The hyphal suspension was streaked in a zigzag fashion using an inoculation loop. Scattered separate spores would have been marked on the back of the plate and transferred to the fresh agar plate after they had germinated (Amare A, 2008). After the incubation period of growth, each fungal colony was subcultured separately on PDA and identified macroscopically based on cultural and microscopic identification using characteristics, such as colony color, growth pattern, growth type, mycelia, and spores. Identification to species level was performed using the color of the mycelium (colony) and the morphology of the conidia, presence of sclerotia, and microscopic examination compared with published literature. Finally, each isolated pathogen was transferred to the PDA slant medium, labeled, and preserved at 4 °C for further analyses.

Morphological and cultural characterization

The isolates of the predominant pathogen(s) had been characterized based on their growth and morphological characteristics. About 15 ml of autoclaved PDA was poured into each sterile Petri dish and allowed to solidify. The colony growth rate of individual isolates, which were collected from different locations, was determined by taking a 5 mm diameter mycelial disc from the edge of the actively-growing pure cultures using cork borer and were placed separately at the center of fresh PDA plates [18]. The treatments were arranged in a completely randomized design (CRD) with three replications and were kept in the incubator at 25 ± 2°C. Colony diameter was measured at an interval of 24 hours starting from the next days after incubating and continued until full growth occurred in the 90mm diameter Petri dish. The measurement was considered as an average of two perpendicular cross diameters. Data on colony color (back side/reverse and upper side/front view of disk), growth pattern (abundant, moderate, and slight), and growth type (fast, medium, and slow growth) of the hyphae/mycelium on culture medium also were recorded. The pathogenicity test of these predominant isolates was conducted in a separate study.

Description of data collected

Colony diameter: The colony diameter of each isolate was measured in millimeters by measuring the diameters of the colony at right angles to each other and taking their average as a linear growth of the isolates.

Colony color: The colony color of each isolate was observed on plates and recorded.

Colony growth rate: the growth of the isolate was measured in millimeters by measuring the diameters of the colony at right angles to each other and taking their average as a linear growth of the isolates within 24 hours of growth.

Growth type: This was described as fast, medium, and slow based on their respective growth duration on the medium. Growth pattern: Described as mycelial growth on the medium as abundant, moderate, and slight growth.

Pathogenicity test

The already isolated and identified fungal isolates were tested in the glasshouse at Haramaya University to investigate the pathogen capacity to induce root rot and the severity of the related symptom on the susceptible (ZABR-16574/21F2Z) accession obtained from Mechara Agricultural Research Center. Sterilized soils were prepared to reduce contamination before inoculation. One kilogram of sterilized soil was filled in each pot with a 12.5 cm mouth diameter. Inocula of the various root rot-causing fungal pathogens were multiplied by plating mycelia on autoclaved PDA medium. The microorganisms grown on the PDA were identified and then transferred to a fresh medium by incubating at 25 °C under darkness for further purification. Pure culture prepared for each pathogen was multiplied on sterilized sorghum grain and incubated for 10-14 days. Then 10 g of fungal isolates grown on sorghum grain were added into the potting soil at a ratio of 1:10 (w/w) and were sown with five (5) common bean seeds per pot. Then the symptomatic plant grown were carefully removed two to three weeks after sowing and maintained for root rot incidence and severity evaluation.

Based on the CIAT visual disease severity scale whose scores varied from 1 to 9 [19], where 1 = no root rot symptoms; 3 = a maximum of 10% of the hypocotyls and root tissues having lesions; 5 = approximately 25% of the hypocotyls and root tissues having lesions and the root system suffering a considerable decay; 7% = 50% of the hypocotyls and root tissues having lesions and the root system affected, 9% = 75% or more

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of the hypocotyls and root tissues having lesions and the root system suffering advanced stages of decay and considerable reduction. Plants classified 2, 4, 6, and 8 were intermediate to the next higher and lower classifications. Isolates that had an average disease score of 1 to 2 were considered as being 'non-pathogenic', while those with an average score of 3 to 5 had been considered as 'moderately pathogenic' and those with an average score of 6 to 9 were considered to be 'highly pathogenic'. In addition to that, isolates were also grouped based on the visual score of their effect on mean emerged seedlings and/or seed germination percentage (0% - 100%) employed by [20] with some modification. Those isolates that infected emerged seedlings (1% to 33%) and inhibited seed germination (67% - 100%) were considered as 'highly pathogenic', (34% to 67%) of both seedling emergence and inhibition of germination were considered as 'moderately pathogenic' and 68 to 100% seedling emergence and 0 to 33% inhibited seed germination were considered as 'less pathogenic'. At the end of the experiment, the pathogens were re-isolated on PDA from the artificially infected plant samples by the standard tissue isolation method. Afterward, the colony characteristics were recorded and compared with that the characteristics of the original isolates to confirm the pathogenicity according to Koch's Postulates. Fungal isolates that proved to cause root rot on common bean seedlings were selected and maintained for further study.

Activity 3. Reactions of Common Bean Genotypes to Aggressive Pathogen Isolates Under Glasshouse

Preparation of inocula for glasshouse experiments

The screening of selected varieties was done in the glasshouse at Haramaya University by arranging treatment combinations. Inocula for glasshouse experiments were first multiplied on sterilized sorghum seed by using the method employed by Tefera, T. and Vidal, S. [21] with some modifications. Sorghum seeds were soaked in water for 12 hours, then autoclaved at 121 °C for 20 minutes and allowed to cool. Agar discs were cut from the pure cultures of selected microorganisms grown on PDA medium and one disc was added into the sterilized sorghum seeds, mixed thoroughly, and allowed to grow for 10 to 14 days. The sorghum seed was thoroughly mixed every three days, under sterile conditions to ensure or confirm complete seed colonization. Ten grams of the infested sorghum seed was spread 1 cm below the soil layer in each pot containing sterilized soil. Ten bean seeds were planted in each pot and finally, the surviving seedlings were thinned to five plants per pot.

Autoclave soil sterilization methods

The soil was disinfected by autoclaving. The procedures used are described as follows: First tap water was added into the autoclave at labeled size, then empty jars were placed in the autoclave. Again soil was added to the tied sack and added to the autoclaved jar by adjusting the autoclave at a temperature in the range of 100-110 °C for two hours. After two hours, the soil was removed from the autoclave carefully and cooled. Then the soil was transported to the glasshouse and subdivided into two-kilogram-sized pots.

Treatments and experimental materials

The inocula of aggressive root rot complex pathogen were applied to the following common bean varieties released by Malkesa ARC and Haramaya University in the glasshouse. A total of twenty varieties of common bean, namely Ado (SAB 736), Tafach (SAB 632), Awash-2, Cranscope, Chorie, Chercher, Argene, Awash-1, SER-119, SER-125, Dendesu, Tinike (RXR-10), Hundene (K-132), Fedis, Babile, Hirna, Kufanziq, Morka (ECAB-0056), and Dursitu, and one susceptible accession as a check (ZABR-16574/21F2Z), obtained from Mechara Agricultural Research Center, were used as treatments and were evaluated. These common bean varieties were newly released and available materials in agricultural research programs but there was no information about their resistance/tolerance or susceptibility against soil-borne diseases for further breeding programs. During the evaluation, seeds sown in uninoculated soil served as a control treatment. Sterilized soil was used in pots as a medium for sowing common bean seeds and inoculating the potential and aggressive pathogen isolates.

Treatment combinations

The treatment combinations were divided along with the twenty common bean varieties mentioned above and aggressive isolate of four pathogen groups (*Fusarium oxysporum* f.sp *phaseoli-F; Macrophomina phaseolina- M, Rhizoctonia solani-R;* and *Sclerotium rolfsii- S*) stated below that re-activated from common bean sample collected (Table 1).

After germination, the seedlings were watered two times per week to provide a favorable environment for the pathogen establishment and development. The survived plants were uprooted two to three weeks after the emergence of the seedlings and were washed with water to remove adhering soil. Evaluation of the disease symptoms was performed on five plants per variety and per isolate with the three replications.

Experimental design and procedures

The Inoculum of the various fungi genera (one aggressive isolate was selected for each identified fungi pathogen) was multiplied by plating mycelia on autoclaved sorghum grains stated under Section 3.3.2. After two weeks of incubation under darkness at 25°C, pre-sterilized soil was mixed with the infested sorghum grains at a ratio of 1:10 w/w in plastic pots. Each pot contained 10 plants of each bean variety used in this evaluation analysis. The pots were set up in a completely randomized design (CRD) with three replications for each aggressive isolate. The inoculum was applied to twenty common bean varieties used as treatments, including the susceptible check. The pots were watered whenever needed.

Disease assessment

Disease incidence was recorded on plants in all inoculated pots. These were done commencing from the first appearance or onset of the symptoms of the disease by taking the percentage of plants showing disease symptoms. Then the plants were carefully removed two to three weeks after sowing and maintained for root rot severity evaluation using a 1 – 9

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Table 1: Treatment combinations of twenty common bean varieties and four aggressive pathogen isolates were used in a glasshouse experiment at Haramaya in 2016

No.	Treatments combinations **						
1	SAB 736 + S	22	SAB 736 + F	42	SAB 736 + M	62	SAB 736 + R
2	SAB 632 + S	23	SAB 632 + F	43	SAB632 + M	63	SAB 632 + R
3	Awash-2 + S	24	Awash-2 + F	44	Awash-2 + M	64	Awash-2 + R
4	Cranscope + S	25	Cranscope + F	45	Cranscope + M	65	Cranscope + R
5	Chorie + S	26	Chorie + F	46	Chorie + M	66	Chorie + R
6	Chercher + S	27	Chercher + F	47	Chercher + M	67	Chercher + R
7	Argene + S	28	Argene + F	48	Argene + M	68	Argene + R
8	Awash 1 + S	29	Awash 1 + F	49	Awash 1 + M	69	Awash 1 + R
9	SER-119 + S	30	SER-119 + F	50	SER-119 + M	70	SER-119 + R
10	SER-125 + S	31	SER-125 + F	51	SER-125 + M	71	SER-125 + R
11	Dendesu + S	32	Dendesu + F	52	Dendesu + M	72	Dendesu + R
12	Tinike + S	33	Tinike + F	53	Tinike + M	73	Tinike + R
13	Hundene + S	34	Hundene + F	54	Hundene + M	74	Hundene + R
14	Fedis + S	35	Fedis + F	55	Fedis + M	75	Fedis + R
15	Babile + S	36	Babile + F	56	Babile + M	76	Babile + R
16	Hirna + S	37	Hirna + F	57	Hirna + M	77	Hirna + R
17	Kufanziq + S	38	Kufanziq + F	58	Kufanziq + M	78	Kufanziq + R
18	Morka + S	39	Morka + F	59	Morka + M	79	Morka + R
19	Dursitu + S	40	Dursitu + F	60	Dursitu + M	80	Dursitu + R
20	Control checks+ S	41	Control checks + F	61	Control checks + M	81	Control checks + R
21	Control/ checks						

** S- *Sclerotium*, F- *Fusarium*, M- *Macrophomina*, R- *Rhizoctonia*.

visual disease score under section (3.2.3.3). Genotypes that had an average disease score of 1 to 3 were considered as being 'resistant', 4 to 6 'intermediate', and 7 to 9 as 'susceptible' as described by Abawi G.S. and Pastor-Corrales M.A. [19].

Data analyses

The percentage data obtained on disease incidence were done using mean comparisons to determine the significantly different variables using Duncan's Multiple Range Test (DMRT). Severity data were recorded by using the CIAT 1 – 9 disease scale. Data were analyzed using Statistical Analysis System (SAS) version 9.2 software (SAS Institute, 2009) and GenStat 15th edition.

Results

Field survey

The recorded data showed that the maximum prevalence (100%) was observed in both altitudinal ranges of 1780-2500 m.a.s.l. and 1360-1779 m.a.s.l. and maximum incidence was recorded in Daro Labu at the altitudinal ranges of 1360-1779 m.a.s.l., whereas minimum prevalence and incidence were recorded in Oda Bultum at altitudinal range less than 1350 m.a.s.l., respectively. Based on the result of their respective soil type, the highest incidence was recorded at Habro on clay-loam and the lowest record was at Oda bultum on sandy soil. Whereas, the maximum and the minimum incidence record was observed on moderate and dry soil moisture respectively (Table 2).

In addition, the maximum incidences were recorded in both sole-cropped and broadcasting common bean fields. While the minimum root rot incidence also was recorded in both row and intercropped fields in all the districts. But the disease was prevalent in all cropping systems and planting patterns under study with varied degrees. According to the data resulting from the field survey and glasshouse this disease was prevalent starting from seedling up to the maturing stage (Figure 2). But, the common bean crop was more prone to root rot disease at seedling up to the flowering stage based on the numbers observed (Table 3).

As per the survey results, the highest plant density group (> 60), was more highly infected than the other group. While the lowest plant density group (10-30) had rarely infected as compared with the rest two groups (Table 4). The sowing date also showed a great variation in the root rot prevalence and incidence across the district and planting time. The highest incidence and prevalence of common bean root rots were recorded under the crop planted during July-August in Daro Labu, and May–June in Habro and Oda Bultum districts. The minimum incidence was recorded in March-April in Daro Labu, and July-August in both Habro and Oda Bultum districts. In most of the surveyed areas, the commonly cropped varieties of common beans were described (Table 4). In these cases, farmers saved and variety bought from the market were exhibiting the maximum root rot incidence in both Habro and Oda Bultum districts. In contrarily, the local variety exhibited the minimum incidence in Daro labu districts (Table 4). During

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Table 2: Common bean root rot prevalence and incidence across altitude, soil type, and soil moisture in West Hararghe Zone during the 2016 main cropping season.

Category	Frequency		Districts									
			Daro Labu		Habro		Oda Bultum		Maximum	Minimum		
	Count	%age	I (%)	P (%)	I(%)	P (%)	I (%)	P (%)	I (%)	P (%)	I (%)	P (%)
1780-2500	7	15.6	8.0	75.0	36.5	100	29.0	97	36.5	100	8.0	75
1360-1779	31	68.9	95.5	100	91.0	96.0	71.6	100	95.5	100	71.6	96
<1350	7	15.6	3.0	83.0	7.5	70.0	18.0	62.0	18	83	3.0	62
Mean (%)			35.5	86.0	45.0	88.7	39.5	86.3				
Sandy	7	15.6	29.9	100	19.9	100	11.3	98	29.9	100	11.3	98
Silty loam	25	55.5	35.5	100	39.0	89	46.3	100	46.3	100	35.5	89
Clay-loam	13	28.9	41.5	100	76.0	100	61.0	100	76.0	100	41.5	*
Mean			35.5	100	45	96.3	39.5	99.3				
Dry	12	26.7	29	45	*	*	*	*	29	45	-	-
Moderate moisture	19	42.2	41.5	96.0	56.0	92.0	47.0	100	56	100	41.5	92
Adequate moisture	14	31.1	36.0	100	34.0	100	32.0	100	36	100	32.0	-
Mean			35.5	72.5	45.0	96.0	39.5	100				

%age= percent observation, I = percent incidence, P = prevalence, *=no numbers

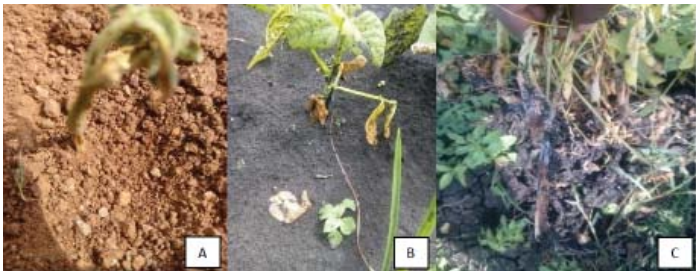

Figure 2: Symptoms of bean root rot in relation to crop growth stage: (A) Seedling stage, (B) Vegetative stage, and (C) Podding stage.

the survey time, the number of major pathogens (*Fusarium*, *Sclerotium*, *Macrophomina*, and *Rhizoctonia*) and insect pests (stem maggot) that had an association with common bean root rot complexes was identified across the surveyed districts. The structures and symptoms of the pathogens on the infected common bean roots and highly infested common bean fields are depicted (Figures 3,4) respectively. During the study, other common bean pests also were recorded and described in Table 4. In this case the highest and lest frequently observed pests were insect and ascochyta blight respectively.

Identification and characterization of common bean root rot pathogen(s)

Sample collection, Isolation, and identification of root rot pathogens: Sixty-nine (69) suspected common bean root rot samples were collected during the survey. From the 69 samples, 27 isolates of different fungal genera were isolated, purified, and identified. The obtained results showed the variation in the frequency of the occurrence among different fungal genera and some nematode spp. in the assayed common bean roots and stem samples. The root fungal pathogens, such as *Fusarium oxysporum* f.sp *phaseoli*, *Sclerotium rolfsii*, *Macrophomina phaseolina* (Syn. *Rhizoctonia bataticola*), and *Rhizoctonia solani* were recorded in high frequency compared with other common saprophytes, such as *Rhizopus solani*, *Aspergillus* spp., and *Trichoderma* spp. The frequency of *Fusarium oxysporum* was recorded as the highest, followed by *Sclerotium rolfsii* and *Macrophomina phaseolina*, having values of 38.39, 21.32, and 14.76%, respectively, in all the assayed samples. Meanwhile, the pathogen referred to as *Rhizoctonia solani* was represented in a less frequency of 10.49% than the other genera related to common bean root rots encountered in the study areas (Table 5). The observation of *Rhizoctonia solani* was maximum in Habro and minimum in both the Daro Labu and Oda Bultum districts of the surveyed area. The assessment results also showed that the mean distributions of isolates of *Fusarium oxysporum* across the districts were high in Daro Labu and Oda Bultum districts, with values of 45.56 and 39.81% respectively, and the minimum observation (29.80%) was recorded in Habro district. The maximum and minimum mean distributions of *Sclerotium rolfsii* were recorded in Habro and Oda Bultum districts with values of 24.11 and 16.74%, respectively. *Macrophomina phaseolina* was also recorded across the districts and was observed with maximum and minimum mean percentages of 16.77 and 12.21% in the Daro Labu and Oda Bultum districts, respectively. In addition to those pathogenic fungi, a number of *Trichoderma* spp. and some nematode spp. were also recorded in the study areas (Table 5).

Morphological and cultural characteristics of common bean root rot causing fungal pathogens: The results of laboratory work showed that eight types of fungal and nematode genera were observed and categorized into two major groups. Those related to common bean root rots were pathogenic, and another group was considered under the saprophytic category. The pathogenic group included *Fusarium oxysporum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseolina*, and Nematode spp. Those considered saprophytes consisted of *Rhizopus*, *Aspergillus*, and *Trichoderma* spp. However, this particular study focused on those grouped under the pathogenic agents related to common bean root rots reported by different authors and again grouped each of them into isolates based on areas of collection and number of samples, and were then characterized.

During laboratory identification twenty-seven pure cultures of different isolates of the genera were prepared for characterization and contaminants were discarded aseptically. Characterization of those isolates was done based on colony color, colony diameter, growth rate, growth pattern, presence of sclerotia, location of sclerotia, and shape of mycelia on growth medium (PDA) and identified the pathogens assisted

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Table 3: Common bean root rot prevalence and incidence in relation to the cropping system, planting pattern, and crop growth stage in West Hararghe Zone during the 2016 main cropping season.

Category	Frequency		Districts									
			Daro Labu		Habro		Oda Bultum		Maximum		Minimum	
	Count	%age	I (%)	P(%)	I(%)	P (%)	I (%)	P (%)	I(%)	P (%)	I (%)	P(%)
S/ cropping	24	53.3	54.0	60.0	66	95	52.0	82.0	66	95	52.0	60
I/cropping	21	46.7	17.0	45.0	24	53	27.0	49.0	27	49	17.0	45
Mean			35.5	52.5	45	74	39.5	65.5				
Row	27	60	24	100	23	64	36	78	36	100	23	64
B/casting	18	40	47	100	67	100	43.0	100	67	100	43	100
Mean	45	100	35.5	100	45	82	39.5	89				
S/ stage	6	13.3	9.5	100	5.6	75	3.1	45	9.5	100	3.1	45
V/ stage	7	15.5	8.9	100	8.8	100	6.9	98	8.9	100	6.9	98
F/ stage	7	15.6	8.7	86	10.0	100	7.7	100	10.03	100	7.7	86
PS/stage	8	17.8	4.5	63	8.4	100	10.8	100	10.8	100	4.5	63
DM/stage	17	37.8	4.1	55	12.2	100	11	98	12.2	100	4.1	55
Mean (%)	45		35.5		45		39.5					

%age= percent observation, I = percent incidence, P = prevalence, *=no minimum, S/ cropping – Sole cropping, I/cropping – Intercropping, B/casting- Broadcasting, S/stage- Seedling stage, V/stage- vegetative stage, F/stage- Flowering stage, PS/stage- Pod setting stage, DM/stage- Dough-Maturing stage

Table 4: Common bean root rot prevalence and incidence in relation to plant density, sowing date, common bean variety, and other pests observed in West Hararghe Zone during the 2016 main cropping season

Variable Category	Frequency		Districts									
			Daro Labu		Habro		Oda Bultum		Maximum		Minimum	
	Count	%age	I(%)	P(%)	I(%)	P(%)	I(%)	P(%)	I (%)	P(%)	I (%)	P(%)
10-30	13	28.9	23	70	20.5	78	17.1	68	23	78	17.1	68
31-60	22	48.9	36.5	92	49.2	96	68.2	100	68.2	100	36.5	92
>60	10	22.2	47	100	65.3	100	33.3	100	65.3	100	33.3	100
Mean			35.5	87.3	45.0	91.3	39.5	89.3				
Mar-Apr	25	55.6	19.5	60	47.6	73	25.5	68	47.6	73	19.5	60
May–Jun	9	20.0	41.0	85	62.4	100	79.0	100	79.0	100	41.0	85
Jul-Aug	11	24.4	46.0	100	25.0	100	14.0	100	46.0	100	14.0	*
Mean	45	100	35.5	81.7	45.0	91	39.5	89.3				
Awas 1	12	26.7	36	100	47	100	42	100	47	100	36	*
Mexican	8	24.4	32	100	52	100	25	100	52	100	25	*
Local	14	31.1	23	59	27	60	54	69	54	69	23	59
Market	11	17.8	51	100	54	100	37.08	100	54	100	37.08	*
Mean	45	100	35.5		45		39.52					
CBB	10	22.2	18.0	38.5	32.0	52.2	27.2	46.3	32.0	52.2	18.0	38.5
Anth	12	26.7	16.0	53.8	60.0	72.1	57.0	69.0	60.0	72.1	16.0	53.8
Ascho	9	20	10.0	23.1	6.0	47.1	2.0	33.3	10.0	47.1	2.0	23.1
Insect	14	31.1	66.8	73.0	95.0	100	100	100	100	100	66.8	73.0
Mean	45	100										

%age= percent observation, I = percent incidence, P = prevalence, *=no minimum, CBB-Common bacterial blight, Anth- Anthracnose, Ascho- Ascochyta blight, Mar-Apr- March-April, May–Jun- May-June, Jul-Aug-July-August

Figure 3: Common bean field infected by root rot fungi in surveyed districts in West Haraghe Zone during the 2016 main cropping season.

Figure 4: Structures of bean root rot pathogen(s) at root horizon in the soil.

by compound microscopic examination or observation. Characteristics of the isolate of *R. solani* were obtained from lesions. On agar plates, the fungus grew very fast and achieved a 90 mm diameter colony in Petri dish cultures four days after inoculation. Those isolates of *R. solani* produced typical

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Table 5: Average frequencies of identified pathogens causing common bean root rots across the districts in West Hararghe Zone during the 2016 main cropping season.

	Average frequency (%)				
Districts	*Fusarium oxysporum*	*Macrophomina phaseolina*	*Rhizoctonia solani*	*Sclerotium rolfsii*	Nematode spp.
Daro Labu	45.56	16.77	7.91	23.10	5.37
Habro	29.80	15.29	15.88	24.11	2.35
O/Bultum	39.81	12.21	7.69	16.74	6.78
Mean	38.39	14.76	10.49	21.32	4.84
Range	29.80- 45.56	12.22-16.77	7.69-15.88	16.74-24.12	2.35-6.8

light gray, white mycelia, dark brown, and both regular and irregularly shaped structures of mycelia on the growth medium. Results of microscopic examination showed that the fungus had typical mycelia of *R. solani*, also septated hyphal branching, both peripheral and aerial sclerotia formation, and lack of pycnidia and spore formation. *Sclerotium rolfsii* isolates produced white, pinkish-white, and milky colors on the medium. The main typical or characteristic feature of this genus is the presence of clamp connection on the hyphal structure and septated type of mycelia, sparsely distributed sclerotia, and sporeless mycelia. Some of the collected isolates of *Sclerotium rolfsii* were cottony, creamy, and brushy like mycelial growth on the upper face of the medium, while some other isolates had both horizontal and slight vertical growth during incubation. Some of the isolates also had very fast growth habits similar to that of *R. solani*.

Macrophomina phaseolina was a common bean root rot-causing fungal pathogen observed in study areas and had light gray color 24 hours after incubation and tended to change to gray 48 hours after incubation, then turned to very dark gray (7.5 YR) color on the upper side of the plate disk. Then 72 hours after incubation, the mycelia became black at the back side of the plate. *Fusarium oxysporum* f.sp. *phaseoli* was a fungal pathogen associated with common bean root rot and had compacted, submerged, and very slow growth at the horizontal direction on the medium. The *Fusarium oxysporum* isolates had dark red (Hue 10R), white and pink at the center, and white at the mycelia tip (Munsell Soil Colour Chart) (Annex 3). Depending on their mean growth rate, the isolates of *Fusarium oxysporum*, *Sclerotium rolfsii*, and *Rhizoctonia solani* showed significant differences at ($p < 0.05$) in the levels of their growth rates and exhibited non-significant differences at ($p > 0.05$) in levels of growth rates over *Macrophomina phaseolina* isolate (Table 6). In *Fusarium oxysporum* isolate, the highest mean growth rate was recorded on the isolated code DL-10-F and the lowest was recorded from the isolate DL-23-F. The isolates of *Sclerotium rolfsii* exhibited the highest mean growth rate, especially the isolated code OB-7-S and the lowest mean was recorded from the isolated code H-5-S. As in the case of *Rhizoctonia solani*, the highest record was observed on the isolated code OB-3-R, and DL-6-R manifested the lowest mycelia growth rate observed on the isolated code H-9-R and H-8-R (Table 6).

According to seven days culture of colony diameter, the isolates of *Fusarium oxysporum* were slow-growing as compared to other isolates of the pathogenic group. All the isolates of *Fusarium oxysporum* could not finish their growth within seven days after incubation. Some of the isolates of *Sclerotium rolfsii* designated by the codes H-5-S and OB-14-S finished their growth within six days after incubation and stopped further radial mycelia growth, while the rest of the isolates finished their growth in five days by producing sclerotia and attained 90 mm diameter but when subtracted (5 mm) diameter of the cork borer, the real or net diameter or size then would be 85 mm. In another case, the isolates coded OB-13-R, OB-14-R, OB-13-RS and H-22-M of *Rhizoctonia solani* and *Macrophomina phaseolina* reached 90 mm diameter within 96 hr or four days after incubation, which is equivalent to 85 mm diameter, while the rest reached 85 mm diameter fifth days after incubation and were considered as fast-growing type isolates (Table 7).

Table 6: Mean growth rates of different isolates of common bean root rot disease complex causing pathogens seven days after incubation (dpi) of cultures.

Isolate genera	Isolate code	Mean growth rate (mm)
Fusarium oxysporum	DL-10-F	23.667^{a}
	DL-3-F	21.952^{ab}
	DL-22-F	21.762^{ab}
	H-22-F	19.071^{b}
	DL-2-F	18.714^{b}
	DL-23-F	18.524^{b}
LSD (0.05)		3.63
CV (%)		9.89
Sclerotium rolfsii	OB-7-S	56.1^{a}
	DL-12-S	52.1^{b}
	OB-2-S	51.5^{b}
	OB-16-S	51.2^{bc}
	OB-14-S	50.6^{bcd}
	OB-18-S	50.6^{bcd}
	H-2-S	49.2^{cd}
	H-5-S	48.5^{d}
LSD (0.05)		2.06
CV (%)		2.33
Macrophomina phaseolina	H-22-M	52.000
	DL-2-M	51.400
	DL-18-M	51.200
	OB-17-M	48.033
LSD (0.05)		5.36 (ns)
CV (%)		5.62
Rhizoctonia solani	OB-3-R	63.810^{a}
	DL-6-R	62.933^{a}
	OB-14-R	61.133^{ab}
	OB-13-RS	58.200^{bc}
	DL-13-R	57.571^{c}
	H-8-R	45.467^{d}
	H-9-R	44.400^{d}
LSD (0.05)		3.22
CV (%)		3.27

Means followed by the same letter(s) within each column are not significantly different at p≤0.05; LSD: Least significant difference at 5% level, CV: Coefficient variation (%).

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Characterization of Common Bean Root rot Disease Causing Pathogens based on Pathogenicity Test: Pathogens that had been isolated from common bean plants displaying root rot symptoms included *Fusarium oxysporum*, *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Macrophomina phaseolina* (Table 5). These all pathogens were tested for their virulence on the susceptible accession, named ZABR 16574/21F2Z, and each isolated genera showed successful infection in all pathogenicity tests. Related to seed and hypocotyls rot as well as cotyledon in the seedlings assay, infected seedlings of common bean showed necrotic lesions, reddish or dark-brown with stem girdling, lesions enlarged to form larger necrotic areas, turned brown and rot, later seedlings eventually dropped out as in case of *R. solani*. Seedlings were rotted, wilted, and died on a scale of 4.3 to 8.6 and were categorized as highly pathogenic disease responses (Table 8). *Sclerotium rolfsii* was recorded as the most highly pathogenic, which was the second most frequently observed pathogen in the study areas during the survey. The pathogen started infection from seed in the soil up to the flowering stage even until the podding stage, especially when parts of the pod contacted the soil surface it become rotted (Figure 2C) from the surveyed field. The white mycelia covered the seeds during germination and seedling growth (Figures 4A and 5A), respectively. When the seed coat was wet, it became softened and the pathogen easily grew on it and dominated the seed before germination; then the seed became invaded and then rotted (Figure 5A). In the seedling assay, the pathogens were grown on roots, infected them, and affected root growth (Figure 4A). The typical symptom of these genera was seed rotting, wilting, and seedling death before two leaf growth stages after germination (Figure 5A and F), the root could not grow well and became stunted. In the potted-seedlings inoculated with these genera, most of the upper soil was covered with sclerotia and a white mass of mycelium within a week. On the contrary, in uninoculated treatment, the seedlings did not show any lesions, or stunting and remained healthy throughout the test period (Figure 6B). *Fusarium oxysporum* was among the pathogens isolated from the common bean crop. In this pathogen, seed emergence ranged between (46.66% - 80%) was observed on inoculated treatment and 95% emerged seedlings observed on uninoculated treatment. Based on uprooted root assay, disease severity reached a 5-8.5 scale (Table 8). Lesions first appeared as water-soaked on the root epidermis, turned brown, and later wilted and then died (Figure 5J, K, and L), suggesting that the fungus was pathogenic on common beans.

There was a very highly significant difference at ($p < 0.001$) in disease severity among isolates of the different genera based on effects on susceptible accession under the study when compared to uninoculated treatment. The isolates of *Sclerotium rolfsii* (coded as H-2-S, OB-16-S, H-5-S, OB-18-S, OB-7-S, OB-2-S, DL-12-S, OB-14-S) that were purified and tested for their pathogenicity were considered as moderately – highly pathogenic, depending on the parameters (mean percentage of rotted seed in the soil, emerged seedling before showing disease symptoms, mean incidence and severity) as well as sclerotia formation (Appendix Table 3) on inoculated body under the study. Depending on its mean percentage of emerged

Table 7: Mean colony diameters after inoculation within 24 hours were measured in millimeters and subtracting (5 mm) diameter of the cork borer.

Iso code	Colony Diameters (mm)						
	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs
DL-10-F	3.67	8.00	13.00	26.67	38.67	44.00	46.67
DL-3-F	3.00	10.33	15.00	23.33	31.00	40.33	45.67
DL-2-F	4.67	9.33	15.67	20.00	28.33	31.33	36.67
DL-22-F	3.33	11.00	20.00	27.67	30.67	35.33	39.33
DL-23-F	3.00	8.33	9.00	23.33	30.00	32.33	38.67
H-22-F	2.83	7.67	9.00	23.33	32.33	36.67	36.67
OB-3-F	3.00	12.32	18.21	25.14	36.00	45.23	49.25
OB-16-S	6.00	29.67	51.67	70.00	85.00		
OB-14-S	3.50	30.00	51.33	66.33	78.00	84.67	
DL-12-S	8.33	32.33	51.67	70.00	85.00		
OB-7-S	9.00	31.67	62.33	83.33	85.00		
OB-18-S	6.00	30.00	49.67	68.00	85.00		
H-2-S	4.33	27.67	48.33	65.00	85.00		
H-5-S	8.00	27.67	46.67	62.00	81.67	85.00	
OB-2-S	5.67	29.33	52.33	71.67	85.00		
OB-17-M	8.83	36.67	63.00	71.67	85.00		
DL-18-M	11.00	41.67	65.00	78.33	85.00		
H-22-M	4.33	41.67	69.00	85.00			
Dl-2-M	8.67	43.33	66.67	78.33	85.00		
OB-13-R	15.00	51.00	80.00	85.00			
H-8-R	8.67	33.00	52.33	73.33	85.00		
H-9-R	9.33	29.33	51.67	71.67	85.00		
OB-3-R	11.67	41.67	70.00	83.33	85.00		
DL-6-R	10.00	36.21	67.00	81.33	84.05		
OB-14-R	8.23	39.33	71.00	85.00			
OB-13-RS	11.00	32.15	79.00	85.00			
DL-13-R	8.33	30.00	54.00	70.67	85.00		

OB-Oda Bultum, H-Habro, DL-Daro Labu, hrs- hours, F- *Fusarium*, S- *Sclerotium*, M- *Macrophomina*, R- *Rhizoctonia*, RS-*Rhizoctonia* from sclerotia

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Figure 5: Pathogenic characteristics of isolates on inoculated susceptible common bean plants on potted seedlings in the greenhouse. Dl-Daro labu, H- Habro, OB- Oda Bultum, S- Sclerotium, F- Fusarium, R-Rhizoctonia, M- Macrophomina

Figure 6: Differences among inoculated and control susceptible common bean potted seedlings for pathogenicity test in the greenhouse.

seedlings, the isolate coded OB-14-S showed less (73.34%) effect on seed emergence and caused less seed rot (26.66%) in the soil (Table 8), whereas the isolate coded with OB-2-S showed less (6.66%) seedling emergence and higher (93.34%) mean percentage of rotted seed in soil (Table 8).

Among the coded isolates of *Fusarium oxysporum* tested for their pathogenicity, OB-3-F, H-22-F, H-10-F, DL-23-F, DL-3-F, DL-13-F, DL-22-F, DL-2-F, and DL-5-F were all pathogenic ranging from moderate to high score on their virulence. The isolates coded as OB-3-F, H-10-F, and DL-13-F scored a mean of 46.66% seedling emergence and caused 53.34% seed rot in the soil. Statistically, it caused about 50% effect on seedling emergence and seed rot. DL-2-F caused less effect on seedling emergence (93.34%) and seed rot (6.66%) (Table 8). *Macrophomina phaseolina* is also one among other identified pathogenic fungal genera known for causing common bean root rot complex. The isolates belonging to this genus were coded as OB-17-M, DL-18-M, DL-24-M, H-1-M, and DL-2-M and tested for their pathogenicity in a glass house and proved their pathogenic abilities on the susceptible accession of common bean. Among those isolates, DL-2-M scored 46.66% mean seedling emergence and caused 53.34% seed rot in the soil and caused a higher effect on the seedling emergence and seed rot than the other isolates as well as the check, whereas the isolate OB-17-M caused less effect on the seedling emergence and seed rot in the soil. Consequently, 80% of the seedlings emerged, and only 20% of the seeds rotted during the pathogenicity test (Table 8). Five *R. solani* isolates coded as H-9-R, DL-19-R, DL-6-R, DL-24-R, and H-8-R were tested for pathogenicity and showed disease symptoms on the inoculated susceptible common bean. Among tested isolates, H-9-R caused a higher effect with mean seedling emergence at only about (13.34%) and seed rot (at 86.66%). The isolate coded as DL-24-R had no effect on seedling emergence and seed rot because 100% of seedlings emerged (Table 8). In addition to the above isolates, there was a combination of all the isolates coded as (RRC) and merged together to study their synergistic effect to produce root rot disease complex on the susceptible accession based on the assumption that in the soil there could be different genera causing root rot of common bean in unison. The result showed that RRC was pathogenic allowing only about 46.66% seedling emergence and causing about 53.34% seed rot (Table 8).

Based on the CIAT severity scale the isolates that had severity scale ranging from 6 to 9 were grouped as 'highly pathogenic' (i.e. H-2-S, OB-16-S, H-5-S, H-9-R, OB-18-S, OB-7-S, OB-2-S, DL-6-R, DL-12-S, OB-3-F, RRCOMP, DL-18-M, H-22-F, DL-24-M, H-1-M, H-10-F, DL-2-M, DL-23-F, DL-3-F, DL-13-F, DL-22-F, H-8-R and DL-5-F) and those that had severity scale ranging from 3 to 5 were grouped

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Table 8: Mean percentage of rotted seeds, emerged seedlings, mean incidence, and severity based on virulence of isolates in a glasshouse experiment.

Isolate Code	Disease Parameters		
	Mean percentage of emerged Seedling (%)	Mean percentage of rotted seed in soil (%)	Severity scale (1-9)
H-2-S	6.66 (1.49)	93.34	9.00
OB-16-S	13.3 (2.98)	86.66	9.00
H-5-S	20.0 (3.59)	80.0	8.80
OB-18-S	20.0 (3.59)	80.0	8.30
OB-7-S	26.7 (4.07)	73.3	8.30
OB-2-S	20.0 (3.16)	80.0	9.00
DL-12-S	40.0 (6.07)	60.0	8.70
OB-14-S	73.3 (8.42)	26.7	4.30
Check	100 (10)	0.0	1.00
LSD(<0.05)	37.39*	37.39*	1.514***
CV (%)	56.8	29.80	10.7
DL-6-R	80.00	20.00	8.60
DL-24-R	100.00	0.00	5.90
H-9-R	13.34	86.66	7.50
DL-19-R	80.00	20.00	4.30
H-8-R	40.00	60.00	8.90
Control check	100.00	0.00	1.00
LSD(<0.05)	62.5	62.5	NS
CV (%)	34.9	56.3	7.0
RRC	46.66	53.34	8.00
OB-3-F	46.66 (6.8)	53.34 (7.27)	8.50
DL-23-F	80.00 (8.8)	20.00 (2.58)	7.10
DL-18-F	66.66 (8.07)	33.34 (5.56)	7.20
H-22-F	60.00 (7.4)	40.00 (6.23)	7.60
DL-24-F	53.34 (6.93)	46.66 (6.8)	8.30
H-10-F	46.66 (5.56)	53.34 (6.9)	7.40
DL-3-F	66.66 (8.07)	33.34 (5.56)	6.30
DL-13-F	46.66 (6.8)	53.34 (7.23)	8.20
DL-22-F	73.34 (8.55)	26.66 (5.09)	7.10
DL-2-F	93.34 (9.6)	6.66 (1.49)	5.00
DL-5-F	66.66 (8.02)	33.34 (4.7)	7.40
Control check	100.00 (10)	0.00 (0)	1.00
LSD(<0.05)	NS	NS	NS
CV (%)	23.6	61.0	27.4
H-1-M	60.00	40.00 (6.32)	6.90
DL-2-M	46.66	53.34 (7.2)	8.60
OB-17-M	80.00	20.00 (3.6)	5.30
Control check	100.00	0.00	1.00
LSD (<0.05)	NS	NS	NS
CV (%)	38.9	41.9	24.6

Means followed by the same letter(s) within each column are not significantly different at p≤0.05, LSD: Least significant difference at 5% level, CV: Coefficient variation (%), *-significant, **- highly significant, DL- Daro Labu, H- Habro, OB- Oda Bultum, F- *Fusarium*, S- *Sclerotium*, M- *Macrophomina*, R- *Rhizoctonia* and RRC- Root Rot Complex

as 'moderately pathogenic' i.e. DL-19-R, OB-17-M, DL-24-R, OB-14-S and DL-2-F. Based on mean seedling emergence, the isolates coded as H-2-S, OB-16-S, H-5-S, H-9-R, OB-18-S, OB-7-S, and OB-2-S were considered as 'highly pathogenic'; the isolates consisting of DL-12-S, RRCOMP, OB-3-F, DL-18-M, H-22-F, DL-24-M, H-10-F, H-1-M, DL-2-M, DL-3-F, DL-13-F, H-8-R, and DL-5-F were considered as 'moderately pathogenic' and the isolates coded with DL-19-R, DL-6-R, OB-17-M, DL-24-R, OB-14-S, DL-23-F, DL-22-F, and DL-2-F were considered as 'less pathogenic'. In other words, those isolates that had a minimum number of mean seedlings emergence had high mean seed rot in the soil (Table 8) and, therefore, they were considered highly pathogenic to the susceptible host.

Pathogenicity and aggressiveness: The pathogenic ability of four representative isolates to induce common bean root rot was tested and all tested fungal isolates were able to provoke root rot at different degrees on a susceptible common bean cultivar. Disease severity that showed how severe the fungi were ranged from 4.3 to 9 according to the CIAT disease scale. *Sclerotium rolfsii* isolate H-2-S motivated a significantly high (9 disease scale) disease severity, while the OB-14-S and DL-19-R isolates caused the least (4.3 scales) severity. Thus, *Sclerotium rolfsii* isolates H-2-S (Figure 5F), OB-3-F (Figure 5K), H-8-R (Figure 5C), and H-1-M (Figure 5I) were more aggressive isolates from each fungal genus, and were selected for the reaction resistance screening on twenty common bean varieties.

Symptoms of common bean root rot: Symptoms observed on infected plants included necrotic lesions on the hypocotyls, main

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and secondary roots; water-soaked areas on the hypocotyls; the collapse of the taproots; decay of secondary roots; brown deep cankers on taproots; brown vascular discoloration; and in some cases, the pink coloration of the hypocotyls, finally wilting and death of the seeds, seedlings and older plants. Soilborne pathogens associated with common bean root rots and these symptoms were *Fusarium oxysporum* f.sp. *phaseoli*, *Sclerotium rolfsii* and *R. solani*. Inoculation with the different pathogens resulted in symptoms similar to those observed on common bean plants growing in naturally-infested soil in the field. Seedlings inoculated with *M. phaseolina* showed dark lesions on the cotyledons and/or stem tissues, seedlings rot above the cotyledon (Figure 5E and I), later wilted, and died. Symptoms observed on seedlings inoculated with *R. solani* were reddish brown, cankers developed on taproots and lower stems, and finally white mycelia with a mass of chocolate brown sclerotia (Figure 4B). *Sclerotium rolfsii* induced water-soaked lesions that subsequently turned dark brown on lower stems and taproot tissues. Later, infected plants wilted, and white cottony-type mycelia were observed on affected tissues (Figure 4A). Poor stands resulted from either lack of germination owing to seed rot or pre-and post-emergence seedling death. The causal fungus infected the base of the seedlings and the lesion girdled the hypocotyls. The fungi produced a fan of silky white mycelium and round sclerotia, which initially were white and gradually darkened. Most of *F. oxysporum* f.sp. *phaseoli* isolate induced stunt growth, seedling stems were thinner than the normal, chlorotic, drying leaf margins to midrib, wilted, and died. A few isolates caused defoliation and wilting of the plants. The vascular systems of these plants showed varied degrees of discoloration. All the isolates under study caused seedling death at early stages, resulting in very poor plant stand as compared to the control plants, which had no symptoms observed at all. Generally, the pathogens responsible for common bean root rot caused nonspecific symptoms, namely stunting, yellowing of leaves, wilting, seedling, and plant death.

Reaction of Common Bean Genotypes against Aggressive Pathogenic Isolates under Glasshouse

Based on the test in glasshouse pathogenicity test, common bean varieties that showed different levels of resistance were identified for testing the reaction of the varieties to the different isolates of the aggressive soilborne pathogens that caused root rots. According to data generated from the glasshouse experiment, out of twenty common bean varieties tested for the purpose, five selected released varieties (Dendesu, Tinike, SER-125, Dursitu, and Chorie), were identified as resistant to *Fusarium oxysporum* f.sp. *phaseoli*, one variety (Chorie) to *Sclerotium rolfsii*, and five varieties (Dursitu, Cranscope, Chorie, Argene, and SAB 632) to *Rhizoctonia solani*, when compared to the genotypic accession used as a susceptible check. Common bean genotypes showed very highly significant ($p < 0.001$) differences in disease severity scale and seedling emergence to both *Fusarium oxysporum* and *Sclerotium rolfsii*, while they did not exhibit any significant ($p > 0.05$) variation in resistance to both *Macrophomina phaseolina* and *Rhizoctonia solani* based on effects on seedling emergence expressed in percentage and effects on root and hypocotyl assay (Table 9). Even if it didn't show any significant difference, there was a high mean variation on their disease severity scale as well as the percent seedling emergence. As a result, Awash 2, which scored 6.7 and 8.2 disease severity scale, was considered highly susceptible to *Fusarium oxysporum* and *Sclerotium rolfsii*, while Dandesu and Chorie varieties that

Table 9: Percent emerged seedlings and disease severity scale for the reaction of common bean genotypes in the glasshouse to four pathogens causing root rot.

Types of genotype	Seed Type	Pathogens							
		F. Oxysporum		S. rolfsii		M. phaseolina		R. Solani	
		PE (%)	DS (1-9)	PE (%)	DS (1-9)	PE (%)	DS (1-9)	PE (%)	DS (1-9)
Tinike	Red Kidney	76.7^{a}	3.0	33.3^{def}	5.9	66.7^{abcdef}	3.8	46.7	5.3
Kufanziq	Pinto	73.3^{ab}	4.0	13.3^{f}	7.5**	73.3^{abcde}	3.7	50.0	4.1
Dendesu	Red	73.3^{ab}	2.9	60.0^{abcde}	4.5	50.0^{cdef}	5.2	36.7	4.5
SER-125	Red	73.3^{ab}	3.0	73.3^{abcd}	4.8	80.0^{ab}	4.2	66.7	4.2
SER-119	Red	73.3^{ab}	3.9	70.0^{abcd}	4.5	63.3^{abcdef}	4.3	63.3	4.6
Awash 1	White	73.3^{ab}	4.2	53.3^{cdef}	5.2	73.3^{abcde}	3.8	36.7	6.1
Babile	Red	73.3^{ab}	3.4	66.7^{abcde}	4.1	50.0^{cdef}	3.6	70.0	4.0
Fedis	Red mottled	70.0^{b}	5.7	46.7^{cdef}	6.4	93.3^{a}	4.5	46.7	5.1
Argene	White	66.7^{bc}	3.9	83.3^{ab}	4.0	63.3^{abcdef}	4.2	80.0	3.3
Hirna	Red	66.7^{bc}	4.9	26.7^{ef}	6.1	86.7^{ab}	3.8	63.3	3.6
Chorie	White	63.3^{bcd}	3.1	96.7^{a}	2.8	70.0^{abcdef}	4.6	83.3	3.2
Morka	Red mottled	53.3^{bcde}	3.6	56.7^{bcde}	5.1	40.0^{ef}	6.1	46.7	3.7
Hundene	Red mottled	50.0^{bcdef}	5.3	60.0^{abcde}	5.0	66.7^{abcdef}	4.8	66.7	3.7
SAB 632	Speckle	40.0^{cdefg}	6.2*	60.0^{abcde}	6.5**	46.7^{edf}	5.1	70.0	3.3
Cranscope	Red Speckle	40^{cdefg}	3.5	40.0^{def}	6.1	33.3^{f}	5.8	53.3	2.7
Chercher	White	36.67^{defg}	5.6	63.3^{abcde}	4.6	66.7^{abcdef}	4.1	63.3	4.7
SAB 736	Large White	30^{efg}	6.5**	63.3^{abcde}	5.2	66.7^{abcdef}	3.6	60.0	4.1
Dursitu	Red	26.7^{efg}	3.1	26.7^{ef}	4.3	40.0^{ef}	3.5	60.0	1.5
Awash-2	White	23.3^{fg}	6.7**	26.7^{ef}	8.2***	60.0^{bcdef}	5.1	56.7	5.8
(check)	Small white	20.0^{g}	4.7	13.3^{f}	4.9	53.3^{cdef}	7.0	40.0	4.3
LSD (0.05)		29.5***	6.3	42.5***	3.2	35.7	2.6	NS	1.1
CV (%)		31.3	21.6	47.8	18.9	33.8	19.8	37.7	23.1

*P<0.05, **P<0.001, Means followed by the same letter(s) within each column are not significantly different at p≤0.05, LSD: Least Significance difference at 5% level, CV: Coefficient variation, *-significant, **- highly significant, PE- percent emerged seedling, DS- Disease severity in scale

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scored 2.9 and 2.8 disease severity scale were considered as resistant to *F. oxysporum* and *S. rolfsii, respectively* (Table 9). As in the case of *Macrophomina phaseolina*, all the common bean varieties that were tested showed intermediate or moderate resistance, except the accession ZABR-16574/21F2Z, which scored 7 on the disease severity scale, was considered highly susceptible and the variety named Dursitu that scored 3.5 and 1.5 showed more comparable resistance than the rest to both *Macrophomina phaseolina* and *Rhizoctonia solani*. Accordingly, Awash 1, which got a disease severity score of 6.13, was considered comparably more susceptible than the rest common bean varieties to *R. solani* (Table 9).

Discussion

During the survey, a total of 45 common bean fields within altitudinal ranges of 1040-2500 m.a.s.l were assessed, of which 48.89% were at the vegetative stage to pod setting stage, 37.78% of the crop was at pod setting to the maturing stage, 13.33% at seedling stage with varied disease incidence and prevalence. In this case, the higher disease incidence was recorded at the seedling stage up to the flowering stage than other growth stages that indicated plants at this stage were more susceptible; but the disease was prevalent at any growth stage. The present finding agrees with the observation of Hagerty (2013) [22] that pathogens are infective at the seedling stage and continue through vegetative and reproductive growth stages. The results also indicated that the incidence of root rot disease varied across districts, altitudes, planting patterns, plant densities, soil moisture, planting dates, cropping systems, crop varieties, soil types, and rainfall. These differences in disease incidence might be due to the variation in the prevailing weather conditions and crop growth stage, which was related to the time of assessment and the number of fields inspected. The current study has similarity to [23] who indicated that bean root rot is favored by long rainfall and fluctuation in soil moisture conditions and Mukankusi, *et al.* [24] has been reported either too little or too much water to escalate root rot symptoms, as both drought and flooding stress predisposes plants to infection. Too much water results in low aeration, which is stressful to plant roots., soil type, and soil texture [25], similarly, low usage of quality seeds by farmers, inappropriate cropping systems, and water logging due to excessive rainfall are considered conducive [26].

Based on the current survey result, high disease incidence was recorded in the clay loam and minimum disease incidence was recorded on sandy soil. It might be that clay loam soil has high water holding capacity which might have indirectly favored root rot- and wilt-causing pathogens whereas the sandy soil had low water holding capacity for a long time. This result disproves the finding of Mathre, *et al.* [27] who observed high common bean root rot disease incidence on sandy soil. The cropping system (sole and intercropped fields) was also recognized to affect the incidence and prevalence of common bean root rot. Intercropping reduced crop lodging, improved soil fertility, and shared the pathogen preference and reduced inoculum load in the soil. In similar line with this current observation, Abawi and Wildmer [28] also pointed out that intercropping improved soil quality and health and directly and indirectly affected soil-borne populations and disease severity. Planting pattern (row and broadcasting), even if the prevalent pathogen was observed in all planting patterns, the highest incidence was recorded only in broadcasting planting patterns. This may be due to the in-row planting system; it may create a less conducive environment for the pathogen to service or perennate in the absence of the susceptible common bean plant. This current finding is in line with the investigation of Kapoor AS, et al, [29] indicated in their survey reports and information on cropping patterns in the farmers' fields that the cropping patterns affected the incidence and severity of common bean root rots. In addition to that, the maximum (100%) prevalence was recorded in all surveyed districts under the group that had plant densities greater than sixty were considered as maximum plant density and this observation is in line with the findings of Burke and Barker [30] who indicated that plant densities affected the prevalence and incidence of common bean root rot since high plant densities can lead to low aeration, high humidity and prolonged periods of dampness.

The major pathogens identified and that were associated with common bean root rot samples collected from the survey districts included *F. oxysporum* f.sp. *phaseoli, S. rolfsii, M. phaseolina*, and *R. solani. Meloidogyne* spp. was also associated with common bean root rots in the surveyed districts of the West Hararghe Zone. Similar observations were done by Ul-Haq, et al. [31] who reported that the diseases can be caused by a single soil-borne pathogen or by a combination of several pathogens, resulting in disease complexes in many countries. During this present survey, all isolated soil-borne fungi were found in all three districts during the assessment. The frequency of their isolation, however, varied from district to district and from cropping time to cropping time, since the districts had varied cropping times. These results agree with other reports on the incidence and severity of root rots in Rwanda [32]. From this current assessment, *F. oxysporum* was frequently isolated and identified from common bean root rots observed; the same pathogen was identified as the causal agent of Fusarium wilt of the common bean by Kendrick and Snyder [33] and Saremi, et al. [34] who reported *Fusarium* spp, as a cosmopolitan root rot pathogen with pronounced economic damage in legumes and cereals. *Fusarium* root rot is specifically caused by *F. solani* f.sp. *phaseoli* and infects the concerned host plant by penetrating the hypocotyl root tissue by mycelial growth resulting from chlamydospores found in the soil and that is favored by hot weather, soil acidity, and poorly fertilized soils and can survive in the soil for years [35]. Despite the relatively high isolation frequency of *Fusarium oxysporum*, most of the symptoms associated with this fungus were stunt growth, thinner seedling stems than the normal ones, chlorosis, drying leaf margins to midrib, wilt, and death. The present research results agree with the findings of Abawi G.S. and Pastor-Corrless M.A. [19] who reported that the aboveground symptoms of the disease included poor emergence and poor stand establishment, damping off, unthrifty growth, chlorosis (especially of lower leaves), wilting, premature defoliation and relatively low yield. Symptoms on roots and lower stem tissues were variable depending on the specific pathogen(s) involved. According to the current survey data and

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the glasshouse results, we observed that common beans are attacked by this soil-borne pathogen at a time from seedling to flowering stage and are more destructive at the seedling stage than at older stages (Figure 2), the finding of which is in line with the investigation of Hagerty (2013)[22] who reported that the fungi can attack the crop during any time from seedling to flowering stage.

Sclerotium rolfsii was the second most abundant species recorded from infected common bean plants in the surveyed areas or districts in West Hararghe Zone. The pathogen showed characteristic features, such as white, radiating abundant mycelial growth on the affected portion of bean plants. Sclerotia began to develop from mycelial growth on the PDA culture medium four to five days after inoculation or incubation. Initially a felty white appearance, but sclerotia quickly melanized to dark-brown coloration, the result of which agrees with the observation of Narasimha (2000) [36] who indicated that initially, sclerotia exhibited white color that later turned to chocolate brown. Based on the present research results, the mean seedling emergence and seed root/germination varied on the susceptible check common bean related to the isolate's virulence. Also, the more virulent isolates decreased the bean seedling emergence rate more than that of the less virulent isolates. These results are compatible with those achieved by Eslami, et al. [37] who evaluated the virulence of *S. rolfsii* isolates on peanuts and identified isolates that were more virulent decreased the peanut seedling's emergence rate more.

Soil-borne pathogens that caused common bean root rot included Macrophomina phaseolina, which was also isolated from the infected samples taken from the crop having different growth stages, and the disease was manifested by forming black mycelia on the roots and stems. The findings agree with the observation of Singh and Singh [38] who reported that overseasoning was facilitated by cushion-like or shaped black sclerotia and primary inocula. Kivisi (2015) [39] also indicated that the pathogen infected the plant at any growth stage, and post-flowering stage; and also the fungus is favored by long rainfall, concurrent heat stress, and fluctuation in soil moisture stress [23]. *Macrophomina phaseolina* was the third frequently observed microorganism associated with common bean root rot and the isolates were tested for their pathogenicity. The pathogen isolates showed pathogenic ability on the susceptible accession and the finding is in line with the observation of Reetha, et al. [40] who reported that *Macrophomina phaseolina* is a phytopathogenic devastating fungus that affects over 500 host crops in the world and has the capability of producing pycnidia and conidia that aid in disease transmission both aerially and associated in crop seeds. It is responsible for many common bean diseases, including charcoal rot, damping-off, ashy stem blight, wilt dry root rot, and it is a root-inhibiting fungus that causes significant losses under high temperatures and drought stresses [41]. *Macrophomina phaseolina* causes adverse effects in areas with erratic rainfall, low soil fertility, and moisture [42]. Songa, et al. [43] and Afouda (2013) [44] reported that Macrophomina is more prevalent in areas under dry conditions with poor soil fertility conditions than in moist and fertile soils.

Rhizoctonia solani was also among common bean root rot-causing pathogens isolated from infected plant samples and stood as the fourth most frequently observed pathogen in survey districts. *R. solani* was an important disease-causing organism capable of infecting plants at most stages of growth from early germination and seedling stage (where pre- and post-emergence deaths could result) through to mature plants where root and stem rots might develop through infection. The pathogen was purified and tested for pathogenicity and exhibited the symptoms related to the disease by *causing seeds*, roots, and hypocotyl rotting, pre- and post-emergence effects on the seed and seedling that showed compatible results with the investigation of Bradley, et al. (2002) and Sikora (2014) [45]who also reported that *R. solani* attacks its hosts when they are in their juvenile stages of developments, such as seeds and seedlings, which are typically found in the soil. Symptoms exhibited included red-brown lesions affecting the hypocotyl at the soil base, wilting, and yellowing of the leaves [46]. It infects the root tissues using propagules, sclerotia, or mycelia [47]. Favorable conditions such as high humidity, and condensed moisture play a greater role in the formation of globe-shaped sclerotia [47]. The pathogen is known to cause serious plant losses by attacking primarily the roots and lower stems of plants. *R. solani* has a wide host range, and is generally air-borne, although cases of seed-borne infection have been reported (Yang and Li, 2012). On agar plates, the fungus grows fast and achieved 90 mm of diameter colony in 4 days after inoculation that confirms the ideas of Rahayu (2014) [48] who reported that the fungus grows fast and achieved 90 mm of diameter colony in 4 days and has been produced typical white mycelia, dark brown and irregularly shaped structure of sclerotia. In this current experiment, it was attempted to evaluate the synergistic effects of all isolated genera where they were incorporated together as (RRc) pathogens, and the final result was pathogenic but not as much as those incorporated solely, the observation of which agrees with the findings of Adandonon (2004) [49] who indicated that when *S. rolfsii* isolates were paired with *F. oxysporum* or *F. script* and incorporated into the soil, the mixture of the pathogens resulted in significantly fewer diseased plants than when the *S. rolfsii* isolates were used separately on their own. The percentage of diseased plants was 75% for the *S. rolfsii* isolate as single inoculum and 60.75% when the isolate was combined with *F. oxysporum*.

In this study, some nematode spp. were also recorded in lesser quantities but not tested for their virulence. From this observation, it was assumed that root rots could be caused by one or more soil-borne pathogens, acting either alone or as a complex of two or more pathogens in unison and the finding is in agreement with the observation of Rusuku et al. [32] who hypothesized that soil-borne diseases caused by either Fusarium spp., Pythium spp. and/or Rhizoctonia spp. are biotic constraints to the common beans production which act either individually or in a complex manner. But, the frequent association of several soilborne pathogens in diseased bean roots needs further investigation to determine whether these organisms cause disease in progression and to what extent synergism occurs. Harveson (2011) [50] reported

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that soil-borne pathogens act as complexes, which affect the root systems of legumes that exhibit more or less the same symptoms, such as superficial or sunken lesions, root, and stem rots, and damping-off on the roots and seedlings, which is at par with this current study.

The role of root and stem-feeding insects was also great in the present study areas. The samples collected from the attacked plants by stem maggots were observed to have more highly rotted plants than by the pathogens alone. This is in agreement with the observation of Ampofo and Massomo (2009) [51] who reported feeding of bean plant various parts, such as leaves, stems by bean stem maggot (BSM), hinder nutrient transport, resulting in wound creation on the plant, thereby opening an avenue for pathogen and generally becoming an entry point for soil-borne pathogens, such as Fusarium, Pythium, Rhizoctonia, and Macrophomina spp. Bean stem maggots (*Ophiomyia* spp.) are recognized as the most important pest of beans in all the production environments in Africa, causing losses of 20% to 100% annually [51]. These vascular pathogens invade the plant via the roots and are likely to be influenced by similar other root rot pathogens. Abawi G. S. and Pastor-Corrless, M.A. [19] has also noted that various combinations of *Pythium* and *Fusarium* spp., *S. rolfsii,* and bean stem maggot (BSM) can occur in beans displaying different levels of plant damage. However, the way in which these organisms interact is unclear. But, Spence (2003) [52] noted that as with root rots, the bean stem maggot (BSM) problem is aggravated by the continuous cropping of common beans in areas with a high human population. Spence (2003) [52] also added that the problem is more acute when BSM and some of the root rot organisms occur together. Management of the disease through varietal screening is an excellent approach to overcoming economic losses caused by pathogens in plants. Although plant disease can also be controlled with chemicals, biological control, and cultural practices, such as crop rotation, tillage, plant density, and clean seeds; resistant varieties tend to be the best options for low-production cost disease control [53]. To initiate the search for resistance to disease, identification of sources of resistance is needed and the development of a technique to screen reputed genotypes is the first step. In this study, an effective and simple screening method developed by [19] was applied to select common bean varieties that display root rot resistance caused by *Fusarium oxysporum*, *S. rolfsii*, *M. phaseolina,* and *R. Solani*. Even though root rot has been studied in several crops, methods of screening for resistance to the pathogen had varied according to the hosts and techniques used [53].

According to our results based on CAIT visual disease severity scale (1-9) [19], there were only two varieties that show resistance characteristics for all tested pathogens while many of the varieties show intermediate resistance for different pathogens that line with Mukankusi (2010)[24] also report in his experiment that, five cultivars had disease severity scores ranging between 4 and 5, while three had severity scores of 5-6 and all these cultivars were considered resistant to the root rot pathogens that occurred, as well as being adaptable. As in our study, some cultivars were highly infected at seedling emergence whereas others were infected at the early vegetative stage. This idea confirms Hall and Phillips (2004) [54] finding, cultivars that appeared to have similar levels of resistance at a young stage differed dramatically at an older stage indicating that resistance of seedlings may not reflect resistance in older plants. From the present study, the highly resistant genotypes could be utilized in the development of common bean verities with resistance to root rot. In general, if this property is confirmed in the case of our study, it should be easily undertaken a national breeding program to improve the level of resistance found in the most popular common bean varieties in Ethiopia. As the resistance to *FOP*, *S. rolfsii, M. phaseolina,* and *R. solani* seems to be effective to various species of these genera, identification of some resistant varieties should constitute a preliminary and basic step prior to undertaking breeding strategies aiming at introgressing the resistance genes in the popular common bean varieties.

Summary and conclusion

West Hararghe Zone is one of the major common bean-producing areas in Ethiopia. However, production of the crop is constrained by many diseases. Root rot complex is one of the major constraints affecting common bean production in the Zone, where further information on the prevalence and incidence of the disease is desired. Moreover, there are gaps in information on soil-borne disease since it is expected to be a minor disease and geographically bounded aspects of the disease as well as specific management of this disease with resistant genotypes are lacking. Therefore, this study was undertaken to assess the incidence, prevalence, identification of the major virulent pathogens associated with common bean root rot, and reaction of the genotypes to the disease. In this test, all the isolates exhibited virulence to the susceptible genotype but with variable levels of their virulence; finally, the most virulent isolates were selected from each genus and checked for genotypic reaction. The pathogens or diseases may damage plants by stressing or killing the root systems. Symptoms observed on infected plants included necrotic lesions on the hypocotyls and main and secondary roots; water-soaked areas on the hypocotyls; the collapse of the taproots; decay of secondary roots; brown buried cankers on taproots; brown vascular discoloration; and in some cases, the pink coloration of the hypocotyls finally wilt and die. In the glasshouse experiment, it was found that some varieties were better resistant than the checks as well as some susceptible varieties showed high degrees of symptoms of the disease since the pathogens could infect the crop at seedling up to the maturing stage.

In conclusion, in West Hararghe Zone, the common bean root rot complex is an important disease that calls for due attention, to economical management using resistant varieties in combination with other possible management options. The determination of variability among *Fusarium oxysporum, S rolfsii*, *M. phaseolina,* and *R. solani* isolates is fundamental to guide the development of appropriate strategies for disease management according to different districts. As there are no reports about the determination of morphological and pathogenic variation,

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the present study provides information on the variability of those pathogens in major common bean growing areas of West Hararghe for the first time. Results would be valuable in developing integrated management strategies for curbing root rot disease

Recommendations

The present study was able to gain significant insight into the presence of root rot fungal pathogens and the threat the diseases pose on the common bean cultivation throughout the West Hararghe Zone. It also implies that some management measures thorough common bean varietal screening against the root rots incited by *F. oxysporum*, *S. rolfsii, M. phaseolina,* and *R. solani* evidenced by the glasshouse test. Hence it is recommended that farmers and investors use common bean varieties: Dendesu, Tinike, SER-125, Dursitu, and Chorie for resistance against *Fusarium oxysporum*, Choire against *S.rolfsii*; Dursitu, Cranscope, Choire, SAB 632 and Argene against *R. solani*, while recommending for experimentation, researchers to use Awash 2 and SAB 736 as susceptible checks to *F. oxysporum*; Awash 2, Kufanzik and SAB 632 to *S. rolfsii*; ZABR-16574/21F2Z to *Macrophomina phaseolina* and Awash 1 to *R. solani*. Therefore, in general perspective, growers should be trained on the wise use of these varieties. Furthermore, growers also need to have access to training programs on cultural methods of root rot disease management which involve row planting, intercropping as it serves to increase pathogen preference, optimizing plant density, timely inspecting field at seedling up to flowering as these stages more prone to the root rot disease, and good quality seed for healthy vigorous seedlings/plants which could help reduce the disease severity. In other cases, the identification of additional sources of resistance should be the focus of the research program. The use of varietal resistance is considered appropriate for small-scale farmers, in preferred and diverse backgrounds. Finally, an integration of genetic and non-genetic options should be a focus of research and extension for the management of root rot complex common bean production for the study area. Determine the relationship of both nematode damage and root rot disease severity of soil-borne fungi to yield of common bean. Further research considerations of root rot complex disease need to focus on the analysis of pathogen diversity and alternative management approaches which are highlighted and underscored by the current research work.

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