

Full Length Research Paper

Assessment and characterization of mung bean (*Vigna radiata*) bacterial brown spot in Eastern Amhara, Ethiopia

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Mung bean is one of the major early maturing pulse crop grown all over the world including Ethiopia. The production of the crop in Ethiopia, however, suffers from many diseases caused by bacteria. The study aims to assess the intensity and identify the major foliar bacterial and fungal pathogens of the crop. Purposively, 3 districts and randomly 90 mung bean fields were surveyed during the study period. Pathogenicity test, macroscopic and microscopic observations and biochemical tests were used for identification. Symptomatic of 33 diseased bacterial samples were initially isolated and purified on nutrient agar. Bacterial brown spot was found as important foliar devastating identified diseases, even if its distribution varied among localities. Water soaked, small, circular, brown lesions surrounded by yellow zones were observed in all bacterial brown spot isolates after 8 days of inoculation. Based on cultural and biochemical characteristics, bacterial isolates were identified as grams negative phytopathogenic bacteria called *Pseudomonas syringae* pv. *Syringae*. However, further characterization of both isolates and phenotypic characteristics of a large population of newly emerged *P. syringae* pv. *Syringae* from various host plants should capture the research attention. This is the first report on the occurrence of such disease in Ethiopia.

Key words: Bacterial brown spot, distribution, Eastern Amhara, identification.

INTRODUCTION

Mung bean is an annual food legume in the subgenus *Ceratotropis* in the genus *Vigna* (Lambrides and Godwin, 2007); it is the seed of *Phaseolus radiates* L., an annual herb of the Leguminosae family (EPP, 2004). It has green skin and is likewise called green bean. It is sweet in flavor

and cold in nature (EPP, 2004). It is a standout amongst the most significant pulse crops, developed from the tropical to subtropical zones around the world (Khan et al., 2012; Kumari et al., 2012). It is a significant wide spreading, herbaceous and annual pulse crop developed

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generally by customary farmers (Ali et al., 2010).

Mung bean is originated from India and it has diversified to East, South, Southeast Asia (China) and some countries in Africa (Imrie and Lawn, 1991). It is a minor crop in Australia, China, Iran, Kenya, Korea, Malaysia, the Middle East, Peru, Taiwan and United States (Itefa, 2016). In Ethiopia, mung bean is a recent introduction and is found in different parts of the country (Teame et al., 2017). It is grown in the north eastern part of Amhara region (North Shewa, Oromia special zone and Southern Wollo), SNNPR (Gofa area) and pocket areas in Oromia region (Hararge) and Gambella (ECXA, 2014). Smallholder farmers in drier environments in Ethiopia grow mung bean. In North Eastern Amhara, farmers in some moisture stress areas have been producing mung bean to supplement their protein needs and to also effectively use scanty rainfall (Asrat et al., 2012). It is also grown in few areas of North Shewa and hence its consumption is not wide-spread like the other pulses. According to CSA (2017), Ethiopia produces about 42915.555 tonnes of mung bean annually and 1.136 t ha⁻¹. From the total annual production Amhara region produces 35297.25 tonnes annually. From the region, North Shewa and South Wollo are known to produce this crop. These two zones produce an average of 26277.54 tonnes annually. Generally, Ethiopia produces less when compared with other countries annual production like India 20.7 million tonnes and others (FAO, 2017). Among pulse crops, mung bean (*Vigna radiata*) is an important short-duration grain pulse crop with wide adaptability, low input requirement and ability to improve the soil by fixing atmospheric nitrogen (Sadeghipour, 2009). It is suited to a large number of cropping systems and constitutes an important source of protein in cereal-based diets (Khattak et al., 2001; Minh, 2014). Mung bean (*V. radiata*) is one of the utmost important edible food legumes of Asia. In India and some South Asian countries, the crop plays important dietary protein source in predominantly cereal rich diets. The crop serves as an alternative source of non-animal protein as was the case in some parts of East Africa during the outbreak of the Rift Valley Fever. Also, it is effectively cooked and does not cause flatulence (Pursglove, 2003). Mung bean has premium quality over other pulses due to its more palatable, highly nutritive, easily digestible and non-flatulent nature (Khan and Malik, 2001).

Recently, domestic consumption of mung bean has increased because of the rising popularity in Ethiopia, cultural foods and perceived health benefits due to high levels of certain minerals and vitamins (Tensay, 2015). In Ethiopia, this crop might be a promising source of human and animal food, especially during winter and summer seasons. It matures quickly and it does not compete with the main winter and summer season crops as wheat (*Trifolium alexandrenum*), sorghum and others. Farmers used mung bean in bordering areas to make the

soil fertile without providing fertilizer on the land. So, farmers regard mung bean as traditional crop and cultivated by traditional farming ((Tensay, 2015). According to this author, farmers in Ethiopia used mung bean as food and fodder (36.8%), income generation, food and fodder (32%), food, fodder and improve soil fertility (22.4%), food, fodder, improve soil fertility and income generation (8.8%).

However, productivity of mung bean is decreased through biotic and abiotic stresses; including diseases, insect pests, drought stress, water stress, extreme high temperature, salinity stress as well as heavy metals (Waniale et al., 2012; Das et al., 2014). Ashrar et al. (2001) considered lower yield potential of mung bean is due to susceptibility to insect pests, diseases, undetermined excessive vegetative growth and small seed size. In Ethiopia, according to Tensay (2015) abiotic factors limiting yields of mung bean in terms of both quality and quantity are extreme drought, cold weather, untimely rain (rain after pod filling) and type of soil used for cultivating it. Biotic factors limiting mung bean productivity includes weeds, leave diseases, flying insects on pod and leave at any growth stage (Tensay, 2015). Chadha (2010) reported that all parts of crop plant including root, stem, branches, petiole, leaves, pods and seeds of the crops are vulnerable to disease and pest.

The most serious fungal diseases which infect the mung bean are root rot (*Macrophomina phaseolina*), web blight, *Rhizoctonia solani* (*Thanatephorus cucumeris*), powdery mildew (*Erysiphe polygoni*), cercospora leaf spot (*Cercospora canescens*), anthracnose (*Colletotrichum lindemuthianum*) (Grewal, 1987). Serious viral diseases include the yellow mosaic viral disease (Karthikeyan et al., 2014) and leaf crinkle virus (Makkouk et al., 2003). It suffers from two major bacterial disease namely, bacterial leaf spot and halo blight (*Pseudomonas syringae* pv. *phaseolicola*) (Jitendra and Anila, 2018).

Among these bacterial brown spot caused by *Pseudomonas syringae* pv. *syringae* in dry bean is the major foliar disease to cause both qualitative and quantitative loss of mung bean (Kavyashree, 2014). It was reported as the most widespread bacterial disease of dry bean in South Africa and yield losses of up to 55% was noticed (Serfontein, 1994). The disease is seed-borne and mainly infects foliage and to a lesser extent, pods. Symptoms of brown spot initially appear as small circular, necrotic (brown) spots on the leaves, often surrounded by a narrow yellow halo (Howard et al., 2016). The reduction in photosynthetic activity and physiological changes are considerable, which leads to potential reduction in yield depending on the stage and time at which the diseases appears. However, the disease intensity depends upon the cultivar, growing period and environmental conditions. The average yield of the crop is limited due to different reasons in Ethiopia EPP (2004).

Understanding the association of disease intensity with

cropping systems, crop combinations and management practices could help to identify the most important variables and to develop an integrated and sustainable management options (Zewde et al., 2007). Furthermore, isolation and diagnosis (verification) of causal agent are very important. However, despite the importance of the disease, there is no information available on the association, distribution and identification of the causal agents in Ethiopia. Particularly, Eastern Amhara National Regional state of South Wollo and North Shewa zones has received little attention from research, development efforts, inputs and no such extensive, quantitative survey and characterization has been done. Knowing such information is crucial to identify the diseases, to map the geographic distribution and determine the status of the disease in addition to providing baseline data to prioritize research problems. On the other hand, such information is of paramount importance as it can be related to yield loss and hence the economic impact of the disease (Ngugi et al., 2002). In addition, the disease rapidly spread and emerged in many mung bean planting areas and is causing a series of mung bean yield losses, making the disease a major threat to mung bean production in the areas. Thus, it is necessary to investigate the geographic distribution of foliar devastating bacterial and disease to know the intensity and characterize the causal agent of the disease. Therefore, the objectives of this study were to: (1) to assess the distribution of bacterial brown spot of mung bean, (2) isolate and characterize bacterial brown spot isolates of mung bean on the basis of cultural and biochemical characteristics and prove pathogenicity of some bacterial brown spot isolates of mung bean at green house.

MATERIALS AND METHODS

Description of the study areas

Field survey was conducted in field of mung bean growing areas during 2018 main growing season in two major mung bean producing zones, namely South Wollo and North Shewa administrative zones of Amhara National Regional state (Figure 1). The administrative district of North Shewa zone in which the survey carried out Kewot is located in the range of 10°41'-11°55' N latitude and 37°20'-39°57'35" E longitude at an altitude of 1120 - 1380 m above sea level (m.a.s.l). The area has an average annual rainfall of 1007 mm, with short rain between March and April and main rainy season between June and September, and annual mean minimum and maximum temperatures of 16.5 and 31°C, respectively (BoA, 2000).

The administrative districts of South Wollo zone for the study area were Kalu and Tehuledere. Kalu is located in the range of 10°55'20"-12°32'6" N latitude and 39°45'43"-39°56'27"E longitude. The climate of Kalu varies from dry sub-humid to semi-arid. The annual average rainfall of the district ranges from 750 to 900 mm. The annual temperature also ranges between 25-35°C. The altitude of the district ranges from 1400 to 2467 m above sea level (KDOA, 2010). Tehuledere district characterized by diverse topography is located in the range of 11°18'21"-11°22'19" N latitude and 39°38'51"-39°44'2"E longitude. The annual temperature varies from

15 to 20°C. The average annual rainfall is 1030 mm. The highest elevated spot of the district reaches 2,928 m.a.s.l. The lowest elevated point has an altitude as high as 1,400 m.a.s.l.

Disease survey and sample collection

A total of 90 fields were surveyed and 15 fields per district were assessed. 33 bacterial samples were considered for the total fields. Incidence and severity were recorded based on the appropriate scales for each disease of each field. The survey was done at two growth stages (vegetative and flowering) of the crop. Geographic features like latitude, longitude and altitude were recorded from all surveyed areas using handheld Global Positioning System (GPS), to trace back the specific locations and symptoms of each disease. Additionally, parameters such as soil condition, cropping system, adjacent crop, previous crop, altitude and growth stage were recorded to determine the relationship with disease intensity. Districts were selected purposively based on intensity of bean production and disease problems, while fields were randomly selected at intervals of about 5-10 km along the main and accessible road sides. Fields were assessed according to Sseruwagi et al. (2004), by walking along in two diagonals (X-Fashion) of mung bean fields at spots of 0.5 m × 0.5 m (0.25 m²) quadrat. Leaf and stem samples were collected as a sampling unit. Disease prevalence was determined by ratio of number of locations showing mung bean diseases to total number of locations/fields assessed for individual diseases and expressed in percentage. Field incidence of each field for each was calculated by totaling the number of plants with symptoms and converting to percent. Diseases severity was rated from 25 selected plants per field, by scoring five representative plants for each five spaced quadrats using standard scales for each disease.

Sample collection was performed immediately after the appearance of the initial symptoms in early August and September in 2018 main growing season. Samples showing bacterial and fungal symptoms during the survey were collected and labeled well in perforated polyethylene bags. Thirty three and twenty five samples showing bacterial and fungal symptoms were taken and transported to Ambo Plant Protection Research Center and the specimens were maintained in refrigerator at 4°C until isolation was carried out.

Disease severity rating

Disease severity rating was evaluated on each plant using a modified 1 to 9 International Center for Tropical Agriculture scale, where 1 = 0% foliage affected, 3=2% foliage affected, 5 = 5% foliage affected, 7 = 10% foliage affected and 9 = 25% foliage affected (Van and Pastor-Corrales, 1987). Further, these scales were converted into Percent Disease Index (PDI) by using formula given by Wheeler (1969).

$$PDI = \frac{\text{Sum of individual rating}}{\text{No. of leaves assessed} \times \text{Maximum disease grade}} \times 100$$

Isolation of bacterial brown spot

Isolation, pathogenicity test and biochemical characterization of BBS isolates were conducted at Ambo Plant Protection Research Center (APPRC). Thirty three samples showing bacterial symptoms taken were grown on the plating media. Nutrient agar (NA) and nutrient broth (NB) were used as plating media (Lelliott and Stead, 1987). Leaf and stem samples were washed with running water thoroughly and approximately, 4 × 7 mm sized small tissue

segments cut from active tissue margins of lesions. Small symptomatic pieces of samples taken from leaves and stems were surface sterilized by 1% sodium hypochlorite solution (laundry bleach) for one minute, 70% alcohol for one minute and rinsed in sterile distilled water three times of one minute each (Geiser et al., 2005; Summerell et al., 2006) and the prepared samples were macerated in sterile distilled water and crashed using laboratory mortar and pestle. Finally, the filtrates were diluted using sterile distilled water and wait for 30 min and allowed the bacteria to float and release from the tissues and then each suspension of the plates were streaked with sterile wire loop on the agar media. The plates were incubated at 30°C for 24 to 48 h; while each plate was examined for culture growth. Those mixed bacterial colonies were purified and grown on nutrient agar. Finally, eighteen bacterial isolates based on similar identity showed on growth media were preserved and used for greenhouse and laboratory analysis. Preserved isolates were stored in 20% glycerol at -80°C.

Identification of bacterial brown spot

Pathogenicity test

Determination of pathogenicity and fulfillment of Koch's postulate is a very important step in the identification of phytopathogenic bacteria. Pathogenicity testing of the obtained isolates were checked by artificial inoculation of leaves of mung bean using the methods described by Lelliott and Stead (1987) and Klement (1990). The test was performed on two week old seedlings of N26, which appears highly susceptible to the pathogen in the affected field. Seeds of N26 variety were provided by Sirinka Agriculture Research Center. Seeds were sown in pots filled with a 3:1:1 mixture of clay soil, sand and FYM respectively as adopted by Suli et al. (2017). Five seeds per pot were sown. The planted pots were placed in the greenhouse with a temperature of 23 to 30°C. For preparing inoculum the representative isolates were cultured on nutrient agar (NA) medium for 24 h. Suli et al. (2017). The inoculum was made by suspending the bacterial cells in sterile tap water to an approximate cell concentration of 1×10^8 CFU/ml measured using McFarland standards at wavelength of 600 nm by ultra-spectrophotometer by the methods stated in Žarko et al. (2017). Inoculation was done with injection of hypodermic needle on the lower surface of leaves and the points of inoculation were sealed with parafilm to prevent entry of external contaminants as reported by Firdous et al. (2009). Similarly control plants were sprayed with sterile distilled water for comparison. After inoculation of plants the relative humidity was maintained by covering the pots with polyethylene bags, watering of the bottom of the pots and sacks placed on the floor of greenhouse. Pots with polyethylene were kept for 96 h. for the bacteria to enter into the tissues of plants. Re-isolation was made after eight days of inoculation and comparison was made between the re-isolated culture and original culture for better confirmation (Jamadar, 1988).

Morphological characterization of bacterial brown spot

As colony morphology on agar surface aids to identify the bacterial isolates, each isolate from colonies of characteristic shape, size and appearance were observed. Characteristic features of the isolate organism were observed by macroscopic (on nutrient agar) and microscopic observations according to Goszczynska et al. (2000) and Aneja (2003). A loopful of culture from overnight grown was streaked on the surface of nutrient agar and was incubated at 30°C for 24 h. Colony morphology, color, texture, margin (consistency) were observed. Shape of the isolate was identified by making simple staining method followed by its observation under

light microscope. Bacterial smear was stained with methylene blue dye and examined. Microbial cells were observed for their shapes like rod or cocci or spiral. Gram staining was performed to look for the gram's nature of the isolates. Purple coloured cells remain gram crystal violet and were called gram positive bacterium. Pink coloured cells lost primary stain and picked up safranin colour and were called as gram negative bacterium (Schaad, 1980).

Biochemical characterization of bacterial brown spot

Nutrient agar (NA) and nutrient broth (NB) were used as plating media (Lelliott and Stead, 1987). Eighteen bacterial brown spot isolates were selected for biochemical tests based on similar morphological and growth characteristics on selective media. These biochemical tests were done three times for better confirmation.

KOH solubility test

KOH solubility test was performed by the method of Fahy and Hayward (1983) using 24 to 48 h old culture. Two drops of 3% KOH placed on to glass slide and the colonies of test pathogen were stirred into the solution clean loop for 5 to 10 seconds. When the solution was viscous enough to stick to the loop, causing a thin strand of thread like slime stretched up, the result recorded as positive. The results were recorded and used for identification of isolates.

Oxidase test

Filter-paper saturated with 1% Kovac's oxidase reagent, (tetra-methyl-p-phenylenediamine dihydrochloride) and placed in a clean Petri dish to look for cytochrome enzymes. A suspected colony of bacteria from NA transferred with wooden stick to the filter paper and rubbed onto the reagent for 30 s. Isolates developed blue or deep purple colours within 30 s were considered as positive for Cytochrome oxidase (York et al., 2004). The results were recorded and used for identification of isolates.

Catalase test

Catalase test was performed by adding 1ml of a 3% solution of hydrogen peroxide to glass slide, a loop of fresh culture grow on NA medium were added into the solution by the method described by Sands (1999). Release of bubble from the culture was recorded as catalase positive. The results were recorded and used for identification of isolates.

Levan production

To test for the levan production of the isolates, a nutrient agar plates containing 5% sucrose were streaked by the test isolates and incubated for 3-5 days at 30°C. until heavy growth occur. Levan produced when colonies were convex, white, domed and mucoid (Fahy and Hayward, 1983). The results were recorded and used for identification of isolates.

Nitrate reduction

Based on the Dickey and Kelman (1988), the ability of the isolates to reduce nitrate to nitrite was evaluated in a test medium that contains NO_3 , 1 g; peptone, 5 g; yeast extract, 3 g and agar, 3 g in 1 L distilled water, sterilized at 120°C for 15 min in tubes. Each

isolate were inoculated by stabbing and sealing with 3 ml sterilized molten agar to avoid false positives and were incubated at 28°C. The growth of each bacterial isolate and bubble formation beneath the upper agar layer was observed and recorded as positive result for nitrate reduction three, five and seven days after inoculation.

Aesculine hydrolysis

Aesculine medium containing plates was streaked by the test isolates and incubated at 20°C. for 2-5 days. Dark colour developed indicates the presence of β -glycosidase activity then, recorded as positive and negative no dark colour developed. The results were recorded and used for identification of isolates (Goszczyńska et al., 2000).

Hydrogen sulfide (H₂S) production

H₂S production was detected according to Aneja (1996) by using sulphide indole motility (SIM) agar medium (peptone, 30 g; beef extract, 3 g; ferrous ammonium sulfate, 0.2 g; sodium thiosulphate, 0.025 g and agar, 3 g in 1 liter distilled water autoclaved at 121°C. for 15 min). The isolates were inoculated by stabbing and by incubating at 28°C. for 48-72 h. The presence of black coloration along the line of stab inoculation were recorded as positive for H₂S production.

Gelatin liquefaction

This test was performed according to methods described by Dickey and Kelman (1988) by employing gelatin medium that contains beef extract, 3 g; peptone, 5g and gelatin, 120 g in 1 L distilled water, poured into test tubes and autoclaved at 121°C. for 15 min and cooled without slanting. The media was stab-inoculated with each isolate grown on YPSA medium for 48 h and that was incubated at 28°C. Three and seven days after incubation, each isolate were evaluated for gelatin liquefaction. The isolates in test tubes were kept at 4°C. for 30 min and gently tipped immediately. A medium that flows readily as the tube is gently tipped was considered as positive for gelatin liquefaction.

Starch hydrolysis

The isolates were streaked on starch agar medium (starch soluble, 20 g; peptone, 5 g; beef extract, 3 g; agar, 15 g in 1 L distilled water with pH 7 and autoclaved at 121°C. for 15 min) to evaluate their ability to hydrolyze starch (amylase production). The plates were incubated at 30°C. for 6 days and starch hydrolysis was observed by flooding the plates with Gram's iodine solution for 30 s. The appearance of clear zone around the line of growth of each isolate indicated starch hydrolysis (Aneja, 1996).

Potato rot test

Fresh potato tubers were washed, alcohol flamed, peeled and sliced into approximately 7 mm width. The slices were placed in 90 mm diameter petri-dishes and sterile distilled water were added to a depth of half the slice. One hundred micro-liters of a 24 h. old bacterial suspension in nutrient broth was pipetted into a 3 mm diameter well on the center of each slice. Positive results was indicated by decaying of potato beyond the point of inoculation; while lack of rotting suggested negative results. Negative control and un-inoculated nutrient broth was used as positive control

(Ignjatov et al., 2007).

Tween 80 hydrolysis

Fatty acid esterase activity was tested by streaking the bacterial cell mass onto a fresh nutrient agar medium containing calcium chloride and Tween 80, a polymer consisting of polyoxy-ethylene-sorbitanmonooleate (Sands, 1990). The medium was prepared from peptone, 10 g; CaCl₂ dihydrochloride, 0.1 g; NaCl, 5 g; agar, 15 g; and distilled water, 1 L; with the pH adjusted to 7.4. Tween 80 was autoclaved separately and added with 10 ml/L and mixed before plating. Incubation was made at 28°C. for up to 7 days (Fahy and Hayward, 1983). An opaque zone of crystals around a colony was recorded as positive reaction for hydrolysis of Tween 80.

Fluorescent and non-fluorescent test

Finally, the above-mentioned Levan, Aesculine, and Gelatine liquefaction were used to differentiate the test isolates into fluorescent and non-fluorescent *Pseudomonas*. The biochemical tests positive reaction were to indicate the fluorescent *Pseudomonas* isolates. Additionally, the fluorescent *Pseudomonas* produces a yellow-green to blue fluorescent pigments on iron-deficient media (KB media). The results were recorded and used for identification of isolates (Goszczyńska et al., 2000).

Carbohydrate utilization

The utilization of Carbohydrates as a sole source of carbon energy is useful in the identification of bacteria particularly *pseudomonads*. Carbohydrate utilization was tested for by using a basal medium and the results were recorded daily for up to 8 days (Hildebrand, 1998). Stab inoculation of bacterial suspension on basal medium containing mannitol, sorbitol, inositol, dulcitol, lactose, sucrose and maltose were tested for their result. Carbon sources were used to distinguish Pss from *P. syringae* pv. *Phaseolicola* isolates. The pH was adjusted to 7.2 with 40% of NaOH. Yellow colour was recorded as positive result.

Data collection and analysis

Data collected from the survey was coded and checked for consistence and completeness and analyzed using SPSS statistical procedure. Descriptive statistics were used to summarize the data. Analysis was conducted by disaggregating important relevant information by districts and zones so that comparison could be made. Pearson correlation coefficient was used to know the association of diseases epidemics and biophysical factors like soil condition, adjacent crop, previous crop and cropping system.

RESULTS AND DISCUSSION

Assessment of bacterial brown spot of mung bean

The results of the field survey conducted at South Wollo and North Shewa zones and laboratory works done at Ambo Plant Protection Research Center are presented in here under. A baseline field survey prevailed that, among the different foliar diseases of mung bean bacterial brown spot caused by *P. syringae* pv. *syringae* was prevalent.

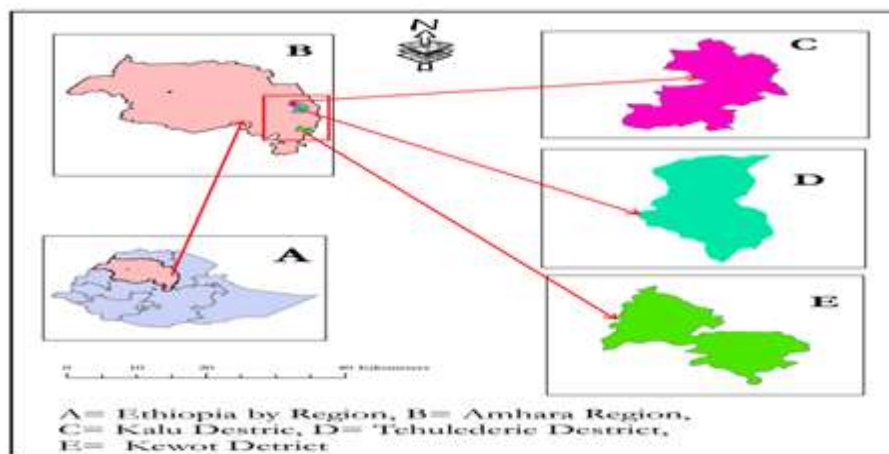


Figure 1. Map of the study areas (Kalu, Tehuledere and Kewot Districts).

Difference in intensity of mung bean diseases in the surveyed belt areas in Eastern Ethiopia was observed. There was variation with respect to disease incidence and severity across the surveyed areas. The variation of diseases intensity in various localities might be attributed to the climatic factors like temperature, relative humidity and distribution and amount of rain fall followed by cultural practices like sanitation and other suitable management practices. Scheuermann et al. (2012) also reported the same result.

The diseases was prevalent and widely distributed in all mung bean growing fields studied regardless of altitude, cropping system, soil condition, previous and adjacent crop. The distribution of this destructive disease in all surveyed areas might be either due to the environmental conditions which promote developments of the disease and/or due to presence of diversified causative pathogen across different mung bean growing areas. Variation in disease prevalence and intensity across locations would be attributed to prevailing environmental conditions and crop management practices, which was reported by Scheuermann et al. (2012).

Likewise, the high disease epidemics saw in all areas may be related to the poor cultural practices adopted by smallholder producers in the areas including utilization of low quality farmer-saved seed sources, absence of harvest turn and poor management practices exacerbated by helpful environmental conditions for disease development. High mean disease intensity observed in all agro-ecologies might be associated to the poor cultural practices adopted by smallholder farmers in the area including use of poor quality farmer-saved seed sources, lack of crop rotation and poor management practices exacerbated by conducive environment conditions for disease development (Njingulula, 2014; Kijana et al., 2017). Dependency on own seed sources could result in the build-up of inoculum and significantly contribute to the development of disease epidemics

(Wachenje, 2002; Mwangombe et al., 2007). The amount of inoculum present in each farm, level of field sanitation and type of cropping system employed might also have influence on the overall disease distribution and epidemics (Stenglein et al., 2003; Mwangombe et al., 2007).

The findings of the study revealed that disease intensity of bacterial brown spot was more pronounced at altitudes greater than 1660 m.a.s.l. This might be partly due to the high rainfall and relative humidity common in the areas, which could favor diseases infection and epidemic development. Muedi et al. (2015) also obtained high disease incidence and severity of bacterial brown spot in altitude ranges of 1350-1735 m.a.s.l. due to high rainfall and relative humidity. The disease was noticed both at vegetative and flowering stages. At stage of vegetative mean incidence of the disease was observed as 45, 39 and 35% at Kewot, Kalu and Tehuledere districts respectively (Figure 2). The mean severities were 15, 12 and 10% from districts of Kewot, Kalu and Tehuledere respectively (Figure 2). The mean prevalence of bacterial brown spot at vegetative stage was recorded as 80, 66.6 and 66.6% at Kewot, Kalu and Tehuledere respectively (Figure 3). At flowering stage, the survey revealed that the bacterial brown spot disease was severe in Kewot and Kalu districts. The mean disease incidence of bacterial brown spot on mung bean showed 80.6, 83.3 and 74% at Kewot, Kalu, and Tehuledere districts respectively at flowering stage (Figure 2). Among districts surveyed, mean maximum severity of bacterial brown spot of mung bean was noticed at Kalu (50.73%) followed by Kewot (42.07%) (Figure 2). The mean least severity of the disease was recorded from Tehuledere district (36.67%) (Figure 2). The mean prevalence of bacterial brown spot was 100 % in all districts surveyed at flowering stage (Figure 3). This implies that the growth stage has a paramount importance for the distribution of the disease.

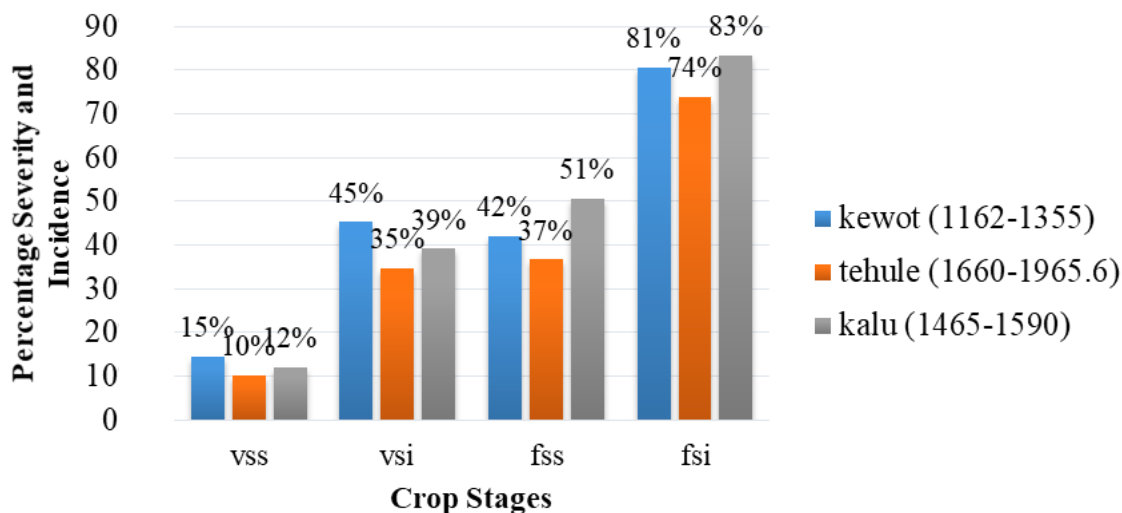


Figure 2. Bacterial brown spot diseases distribution with growth stages in the study districts with respect to altitudinal ranges.

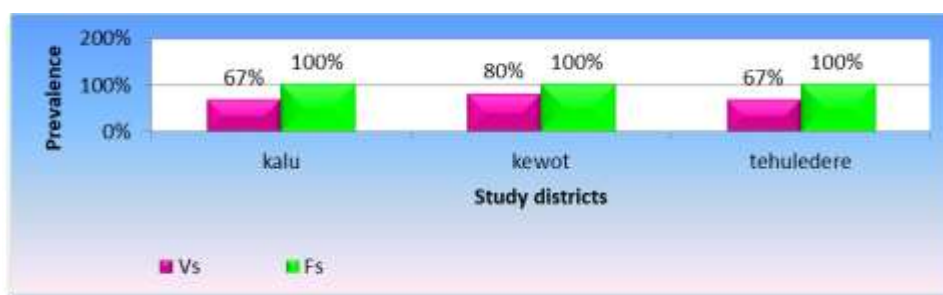


Figure 3. Prevalence of bacterial brown spot in the study areas at vegetative and flowering stages. Vs = Vegetative stage; Fs = Flowering stage.

Some of the mung bean growers were used chemical spray as diseases management option, but some growers were found to apply cultural practices like rouging and cutting of diseased plants and plant parts as disease management schemes to reduce rapid pathogen dispersal among plant canopies. Most of the farmers do not use pesticides as well as other protective mechanisms to prevent the negative impacts of pests and diseases associated with producing the mung bean. About 97% of the farmers were found not using pesticides for the management of bacterial brown spot at vegetative stage and 45% of growers were found using pesticides for the management of diseases with having no awareness on the type of pesticides to be applied (Table 1). Eighty eight percent of the grower's field were weed infested and 11% of fields were weed free (Table 1). With regard to cropping system, 88% of inspected farms were sole cropped and the remaining 12 % was intercropped with maize and sorghum (Table 1).

Regarding to previous crop, 13, 28 and 57% of fields

were observed to have fallow, pulses and cereals respectively (Table 1). About (64%) of the adjacent crops observed in accordance with mung bean fields were cereals (Table 1). The rests (15%) and (20%) of fields were pulses and fields with no crops (Table 1). Some other mung bean disease and insect pest were also observed in association with the above diseases. Yellow mosaic virus and foliage beetle (*Ootheca* spp.) of mung bean were observed in all districts.

Association between biophysical factors and disease parameters

The correlation coefficient (r) between bacterial brown spot severity, incidence and variables like planting stage (vegetative-flowering), previous crop (fallow-cereal-pulse), soil condition (wet to dry), altitude (from low to high), adjacent crop (pulse- cereal-none) and cropping system (mono-intercropping) showed a relationship at

Table 1. Number of fields observed with different cultural practices.

Cultural practices	Number of fields observed in %
Pesticide used at vegetative stage	97.8
Inter-cropped fields	22.2
Sole-cropped fields	12.2
Fallow lands	13.3
Previous cereals	57.9
Previous pulses	28.8
Weed infested fields	88.8
Adjacent fields with pulses	15.5
Adjacent fields with no crops	20.01
Pesticide used at flowering stage	45
Adjacent fields with cereals	64.4
Fields with not weed infested	11

vegetative and flowering stages. Except growth stage and previous crop at flowering stage as well as previous crop at vegetative stage, the other variables association were negative with disease parameters at both stages (Table 4 and 5). Growth stage and previous crop showed positive association with disease severity and incidence at flowering stage (Table 4) and also previous crop showed a strong positive association with disease intensity at vegetative stage (Table 5).

Positive association between bacterial brown spot epidemics and growth stage has been observed at flowering stage (Table 4). This indicates that growth stage might have an influence to the disease epidemics. This might be because of the expansion in leaf canopy, due to the important relative humidity for the advancement of bacterial brown spot. Similarly, Robert (2009) directed the advancement of bacterial brown spot during the mid-vegetative to early flowering stage is because of the relative moistness due to expanded covering.

Positive association of previous crop at both stages with disease severity might be due to the pathogen ability to have a resident phase on both host and bean crop residue that may be an overwintering source for the pathogen (Tables 4 and 5). The epiphytic nature of the pathogen on weeds could result to grow and survive on the main host. Pathogen with a large host range has an increased chance of survival so that some plant pathogens may survive in alternate hosts without causing disease until they come in contact with the main host. Robert (2009) also reported the same finding with this result.

Negative connection of cropping system with disease severity for both stages might be attributed from the increase in spatial distance between host plants, which might inhibit free dispersal of pathogen and suppress weeds responsible for the build-up of high humidity under the canopy (Tables 4 and 5). It might also be due to

rotating crops with non-host crop prevents the buildup of large population of pathogens. The factors result from the system itself and include change in microclimate, reduced host density, induced resistance and competition. Each of the factors may have a minor role in affecting disease. In the system, because of shading by the associate crop, temperature is relatively lower which is known to delay development of BBS. Thus, the bacterial multiplication and movement within the plant cells could be reduced and lower the inoculum. This finding is in line with Muedi et al. (2015) who stated that bacterial brown spot is prevalent where dry bean monocropping was practiced. There are also several studies with regard to the inhibition potential of intercrops against different pathogens in various pathosystems (Chemeda, 2003; Altieri et al., 2005; Habtamu et al., 2015).

The association of disease severity and adjacent crop showed negative and significant association at flowering and vegetative stage respectively at significance level (Tables 4 and 5). It might be due to the unavailability of diseased plants which provide the inoculum responsible for disease outbreaks in nearby bean fields. The absence of plants close to other fields that were infected in the past could be partly attributed for low disease epidemics. According to Ocimati et al. (2018), alternate and volunteer hosts in the neighboring fields are the source of inoculums for bacterial brown spot outbreaks.

Although altitude showed negative relationship with intensities of disease at both growth stages, it was not significant (Tables 4 and 5). It might be due to the suitable environmental conditions favoring BBS development and/or there might be variable pathogen races in the surveyed areas, which enable the pathogen to widely infect the host regardless of elevation differences. It could be also due to ideal moderate to warm temperature as favor for epidemics and severe localized disease outbreaks of bacterial brown spot. If environmental conditions are known to favor disease

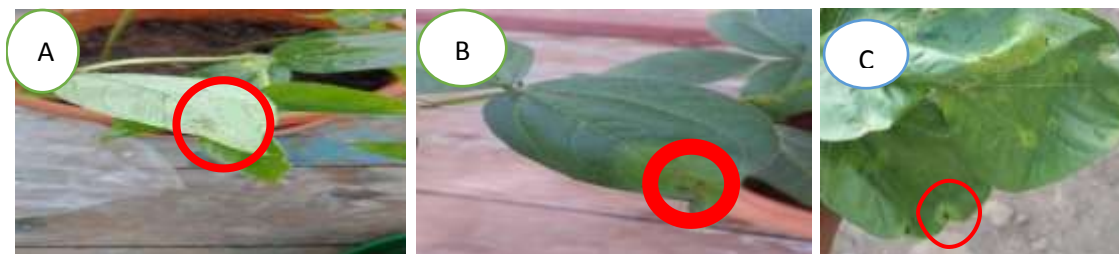


Figure 4. Water soaked on the underside of the leaves, (B&C): small, circular, brown lesions surrounded by yellow zones at green house and field condition.

development, there might be high disease incidence and severity even in high altitude areas ranging 1625-2000 (Muedi et al., 2015). Schwartz et al. (2019) also reported the incidence and severity of bacterial diseases such as halo blight and bacterial brown spot outbreaks are most serious when temperatures are moderately cool and humidity is high.

Soil condition relation with disease severity directed negative connection at flowering stage and vegetative stage (Table 4 and 5) that might be due the presence of dry soil condition during the assessment time for the spread of bacterial brown spot of mung bean. Indirectly wet soil is good for the spread of bacterial brown spot of mung bean. The same result has been reported by Muedi et al. (2015) who stated that avoiding working when soil is wet will decrease the disease severity and development as well as working when the soil is dry, which could result in reduction of disease epidemics.

Symptomatology of bacterial brown spot

Based on this study macro and microscopic observations, pathogenicity and biochemical tests revealed the pathogen called as *P. syringae* pv. *Syringae* (Pss) that leads the disease called bacterial brown spot of mung bean, which is newly emerging disease of the crop. *P. syringae* pv. *syringae* (Pss) has a wide host range groups even in different genera, infecting a number of host plant species. The typical leaf spot symptoms by all isolates were observed on leaves after eight days of inoculation. The symptoms of the leaf spot disease were characterized by production of small circular brown lesions surrounded by yellow zones (Figure 4B and C) and evidence of water soaking was visible on the underside of the leaves (Figure 4A). Linear necrotic lesions among veins were formed as a result of coalescing of lesions. On the affected leaves old lesion center fall out leaving shot holes was observed.

Bacterial brown spot is the most economically important diseases of the processing beans and woody plants Goszczynska et al. (2000). It was commonly reported on dry bean and stone fruit trees as *P. syringae* pv. *Syringae* (Pss). Brown spot of bean (*Phaseolus*

vulgaris L.) (Goszczynska et al., 2000; Serfontein, 1994), bacterial canker of stone fruit trees, bacterial blossom blight or blast of pear and citrus blast and black pith (Goszczynska et al., 2000) were noticed. Saettler (2005) reported the disease has wide host ranges, infecting more than 180 host plant species including woody plants and weeds.

In line with this Howard et al. (2016) showed initial symptoms of brown spot first appear as minor rounded, necrotic (brown) spots on the leaves, often encircled by a fine yellow halo. Similarly Hagedorn and Inglis (1986) noticed symptoms may first look like as water-soaked spots, which gradually enlarge and dry up, and are often bordered by a narrow yellow or light green zone. Wounds may unite and occasionally abscise, later giving the foliage a ragged appearance. According to Watson (1980), bacterial brown spot leaf symptoms on beans are small, irregular necrotic lesions that are sometimes surrounded by a narrow, pale green chlorotic zone. Lesions may coalesce, dry out and become brittle, giving leaves a tattered appearance.

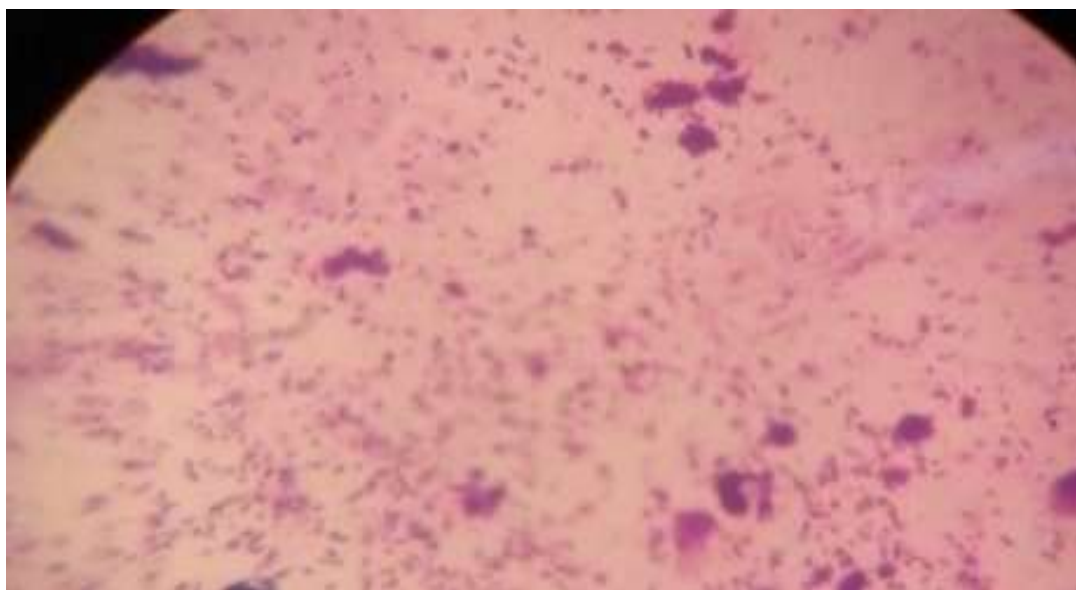
Morphological and biochemical test results of bacterial brown spot

The characterization of phytopathogenic bacteria helps to know the target pathogen and its biological behaviors. Microscopic observations like shape and grams nature revealed the availability of similar morphological characters among isolates. Growth of bacterial colonies after 48 h under aerobic conditions at 30°C resulted in round, yellow with complete margins, rod shaped and mucoid appearances (Table 2). The gram staining method revealed that test isolates showed rod shaped and gram negative bacteria (Figure 5). KOH test confirmed that all the isolates showed positive reaction and were categorized as gram negative rod shaped phytopathogenic bacteria.

The isolates were negative for oxidase reaction, starch hydrolysis (Figure 7), potato soft rot, hydrogen sulfide (H₂S) production, lactose tests and were positive for KOH (Figure 10), catalase (Figure 11), Aesculine (Figure 6), iron-deficient media (Figure 8), tween 80 hydrolysis

Table 2. Macro and microscopic characteristics of bacterial brown spot isolates.

Number	Isolate code	Colony color	Colony shape	Colony texture	Colony margin	Cell shape
1	keF14	Yellow	Round	Mucoid	Entire	Road
2	keF11	Yellow	Round	Mucoid	Entire	Road
3	Ka14	Yellow	Round	Mucoid	Entire	Road
4	KeF8	Yellow	Round	Mucoid	Entire	Road
5	Tf1	Yellow	Round	Mucoid	Entire	Road
6	KeF1	Yellow	Round	Mucoid	Entire	Road
7	Kaf2	Yellow	Round	Mucoid	Entire	Road
8	KeF5	Yellow	Round	Mucoid	Entire	Road
9	KeF2	Yellow	Round	Mucoid	Entire	Road
10	KeF7	Yellow	Round	Mucoid	Entire	Road
11	Kef6	Yellow	Round	Mucoid	Entire	Road
12	Tf3	Yellow	Round	Mucoid	Entire	Road
13	Ka15	Yellow	Round	Mucoid	Entire	Road
14	KeF13	Yellow	Round	Mucoid	Entire	Road
15	KaF6	Yellow	Round	Mucoid	Entire	Road
16	Tf13	Yellow	Round	Mucoid	Entire	Road
17	Ka10	Yellow	Round	Mucoid	Entire	Road
18	Tdf9	Yellow	Round	Mucoid	Entire	Road

**Figure 5.** Morphology of rod shaped gram negative bacteria.

(Figure 9), gelatin liquefaction, nitrate reduction, levan production, sucrose, mannitol, sorbitol, maltose, inositol and dulcitol tests (Table 3). Carbon sources were used to distinguish *Pss* from *P. savastanoi* pv. *Phaseolicola* isolates. Finally, the isolates were identified and considered as *P. syringae* pv. *syringae* on KBC selective media in combination with other biochemical tests conducted to identify the target pathogen.

Consistent with this, Goszczynska et al. (2000) reported

that bacterial brown spot isolates are light cream with entire margins, rod and fluorescent. According to Wazeer et al. (2014) growth of bacterial colonies after 72 h under aerobic conditions at $28 \pm 2^\circ\text{C}$ resulted round, yellow with complete margins, dome shaped, shiny, smooth and mucoid appearances. Bacterial morphological features alone are of little taxonomic value; because they are too simple to provide enough taxonomic information (Lelliott and Stead, 1987).

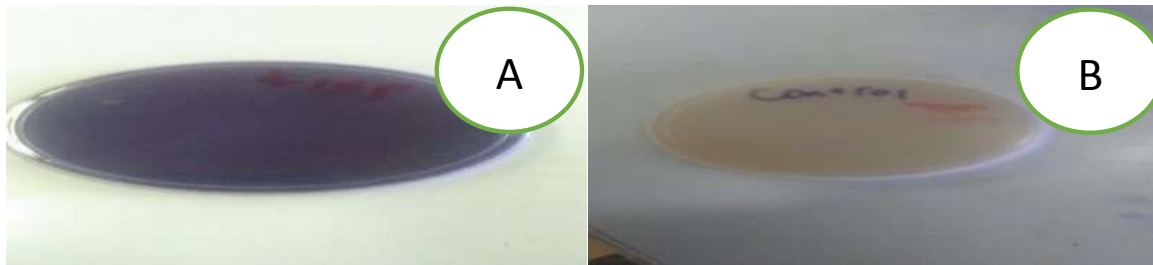


Figure 6. (A&B). Positive for aesculine test and aesculine control on NA.



Figure 7. Negative for Starch test (left) and control (right).



Figure 8. Growth of bacterial brown spot isolate on KB (left) and NA media (right).



Figure 9. Opaque zone around colony recorded as positive reaction for hydrolysis of Tween 80.



Figure 10. Positive for KOH test.



Figure 11. Positive for catalase result.

Similarly, Muedi et al. (2011) stated all fluorescent isolates tested were levan-positive, oxidase -negative, potato soft rot-negative, arginine dihydrolase-negative and mannitol-positive, sorbitol- positive, inositol- positive and carbon sources were used to distinguish Pss from *P. savastanoi* pv. *Phaseolicola* isolates. The result is also in agreement with results obtained by Mohammad (2013) who directed bacterial brown isolates are negative for grams reaction, oxidase reaction, nitrate reduction, starch hydrolysis, lactose, hydrogen sulphide production and positive for gelatin, aesculine hydrolysis, levan production and utilization of carbohydrates.

According to Žarko et al. (2017) report, *P. syringae* pv. *syringae* isolates are positive for levan production, aesculine hydrolysis, gelatin liquefaction and negative for oxidase reaction, potato soft rot, arginine dihydrolase production, tyrosinase activity and tartarate utilization.

Wazeer et al. (2014) reported that *P. syringae* pv. *syringae* (Pss) isolates are positive for mannitol, aesculine, gelatin and negative for arginine and nitrate reduction.

Conclusions

In the present work, field survey, observation and laboratory diagnosis were performed to investigate the status, isolate and identify the causal pathogen. In the present work, we have performed field survey, observation and laboratory diagnosis to investigate the status, isolate and identify the causal pathogen.

In the present work, we have performed field survey, observation and laboratory diagnosis to investigate the

Table 3. Biochemical test results of bacterial brown spot isolates.

S/N	Isolate code	Biochemical tests																		
		KOH	H ₂ O ₂	Oxidase	Starch	Potato sr	Aesculineeee	Nitrate Red ^d	Gelatin	Lactose	Sucrose	KB	Tween80 Hydrolysis	Levan	H ₂ S prd ^d	Mannitol	Sorbitol	Inositol	Dulcitol	Maltose
1	keF14	+	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+
2	keF11	+	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+
3	Ka14	+	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+
4	KeF8	+	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+
5	Tf1	+	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+
6	KeF1	+	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+
7	Ka2	+	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+
8	KeF5	+	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+
9	KeF2	+	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+
10	KeF7	+	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+
11	Kef6	+	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+
12	Tf3	+	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+
13	Ka15	+	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+
14	KeF13	+	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+
15	KaF6	+	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+
16	Tf13	+	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+
17	Ka10	+	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+
18	Tdf9	+	+	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+

status, isolate and identify the causal pathogen bacterial brown spot of mungbean. In Ethiopia, mung bean is a recently introduced crop. It is a niche for several foliar pathogens. Thus, field study conducted to determine the distribution and association of bacterial brown spot disease of mung bean with different biophysical factors and laboratory diagnosis done to isolate and identify the causal pathogens revealed the disease as

bacterial brown spot (*P. syringae* pv. *syringae*). Additional pests like yellow mosaic virus and foliage beetle were observed in combination with the above diseases.

Accordingly, the disease were prevalent and widely distributed. This indicates the favorable environmental conditions coupled with cultivation of susceptible mung bean cultivars and non-implementation of appropriate management

practices worsened the problem to maximum. Bacterial brown spot were found as major ones that limits the production of the crop. This implies that appropriate interventions are needed through creating awareness for growers about disease causative agent characteristics, survival, dispersal as well as possible management options to reduce effects below economic injury level. The intensity of disease observed also

varied between districts and altitudes. This might be due to differences in weather and management practices applied by farmers during the survey year. This directs worthwhile of conducting similar assessments in different mung bean belt areas of the country for all diseases.

The independent variables such as soil condition, cropping system, growth stage, previous crop and adjacent crop showed association to disease severity with different significance among them. Thus, the disease was favoured by warm to hot semi-arid with mid high land, moist conditions and lush crop canopies. This shows role of weather conditions and agronomic practices are under considerations in the epidemics and severities of the disease advancement. Based on the laboratory diagnosis a newly emerging disease of mung bean named as bacterial brown spot of mung bean (*P. syringae* pv. *syringae*) were identified. This implies more isolates covering wide agro-ecologies shall be collected and identified further. Result from pathogenicity test revealed that all tested isolates were symptomatic to the inoculated susceptible variety. The production of typical symptoms for bacterial brown spot was observed under greenhouse condition of controlled environment. This implies that there is an urgent need to study the aggressiveness of isolates of the crop.

Recommendations

This study showed the current status of major mung bean disease in the study areas, identified to be bacterial foliar disease in growing areas of North Shewa and South Wollo zones of Ethiopia. At present, this disease threatens mung bean production.

Therefore, based on this study the following are suggested or recommended:

- (i) Survey should be undertaken to know the distribution of this disease and other pests across the producing regions of Ethiopia.
- (ii) Role of weather conditions in the epidemics and severities of the disease may be investigated through correlations and environmental models as it was not much studied in the present study.
- (iii) This study could provide first information on morphological and biochemical characterization of bacterial brown spot of mung bean in North Shewa and South Wollo growing zones of Ethiopia and it is useful for researchers to go on further molecular characterization studies of isolates.
- (iv) It would be more interesting to study phenotypic characteristics of a large population of newly emerged Pss from various host plants in different regions of Ethiopia. Further collection, isolating and identification work should be sustained.
- (v) Awareness creation about diseases is very necessary for farmers, development agents and others commercial

producers since the problem is not well popular and understood by the mung bean producers and partners.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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