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SCREENING OF IMPROVED AND LOCAL CULTIVARS OF POTATO FOR RESISTANCE TO POTATO LATE BLIGHT DISEASE (Phytophthora Infestans)

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ABSTRACT

Potato late blight disease caused by Phytophthora infestans has become an economic disease in the world and particularly in potato growing regions of Cameroon. Four improved (Pamina, Sassy, Desiree, Diaman) and two local (Belo, Banso) cultivars of potatoes were screen in the field and screen house in Regional College of Agriculture and Catholic University laboratory for resistance to potatoes late blight disease (PLBD). These potatoes cultivars were planted on four ridges per plot in the field in randomized complete block design with three replicate. These cultivars were monitored for disease incidence and severity and data was collected. Potatoes cultivars were planted in a screen house and inoculated with spore suspension of P. infestans of 2.5×104 spores / ml of distilled water to evaluate the resistance of the various potatoes cultivars. Screen house and field screening showed significant varietal difference ($P \leq 0.05$) in sensitivity to the fungus. The improved and local cultivars of potatoes from field and screen house show varying levels of resistance to (PLBD). All the improved potatoes cultivars (Pamina, Sassy, Desiree and Diaman) were highly susceptible to (PLBD) compared to the local cultivars (Belo, Banso) and improved cultivar Desiree that were susceptible. The highest mean disease severity of (PLBD) of 5.0 % was recorded on cultivars, Pamina, Sassy and Diaman at 12 weeks after planting. Diaman recorded the highest mean area of (PLBD) growth of 11.45 m2 in the screen house experiment. The local and improved potatoes cultivars that were susceptible could be planted in the field by farmer to reduced disease incidence and severity and increase productivity of the crop.

Keywords: Screening, improved, local, potato, resistance, late blight, disease, Phytophthora infestans.

1. INTRODUCTION

Potato (*Solanum tuberosum* L) is one of the important food crops in the sub-Saharan region of Africa (MINSERI-IRAD, 2012). The potato occupies the fourth place after the wheat, rice and corn crops. It is therefore considered as the non-main cereal foodstuff in the world (FAO, 2009). Potato is a very important crop in the world, grown in tropics, subtropics, temperate zones and broad agro-ecological conditions from 100 m to 4000 m altitude (Dahal and Rijal, 2019; FAO, 2008) . Many people consumed potatoes as their main food source. In Cameroon, potato is an important source of income, because part of its production is sold in the local market or exported to the neighbouring countries. It is eaten in various forms such as boiled, pounded, chips and also

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used in industries to process animal feed (Ekin ,2011; King, 2013; Izmirlioglu and Demirci ,2015; Jagatee *et al*., 2015)

In Cameroon, potato is produced in areas of high altitudes, especially by small farmers (IRAD, 2012). Potatoes is cultivated in six regions of Cameroon which are, the North-West, the South West, the West, Adamawa, Littoral and the Far -North (Fontem *et al*.,2005). National production of potatoes was estimated to be 229000 tones over 23500 hectares in 2009 (IRAD, 2012). The climate and ecological diversity of Cameroon is favourable for the growth and development of plant and also conducive for the growth of plant pathogens which causes disease in plants including potatoes thus reduces productivity (Yengoh*et al*., 2011).

The most common disease that affects potato in Cameroon is late blight disease cause by the oomycete pathogen *Phytophthora infestans* (Fry, 2008). Late blight infection is characterized by large, dark brown or black lesions often appearing greasy on stems, petioles and leaves of disease plants (Weisz *et al*., 1994). Late blight may cause total destruction of all plants parts (leaves, stems and tubers) in a field within a week or two when weather is cool and wet (Mercure, 1998). The disease destroyed the foliage and stems of potato plant during the growing season. It also attacks potato tubers causing rots in the field or in storage (Agrios, 2005). In Cameroon potato yields are generally low due to an increase rate of late blight infestation (Fontem and Aighewi, 1993). High yielding varieties of potato (Cipira and Tubira) proposed by the International Potato Center (CIP) and Institute of Agricultural Research for Development (IRAD) for production in Cameroon have lost their performances and have become susceptible to diseases (Njualem *et al*., 2001). Yields reduction due to late blight disease varies from 3.3–6.7 t/ha and do not satisfy the needs (Njualem, 2010).

The increase in field problems of late blight disease has led to renewed research efforts geared towards the identification of sources of resistance and their incorporation into breeding populations for crop improvement. The originality of this study was aimed at identifying a reliable, rapid and simple screening technique for evaluating improved and local cultivars of potato for resistance to potato late blight disease (PLBD) and to establish a relationship between screen house and field behaviours of potato host plants towards late blight disease.

2.MATERIALS AND METHODS

2.1 Location of experimental site

The study was conducted in the Catholic University of Bamenda Laboratory, Northwest Region of Cameroon. The positions of the experimental site were recorded using GPS mark Garmin etrex 20. Catholic University is located between latitude 50˚, 95'N and 60˚, 01'N, longitude 100˚, 13' E and 100˚, 26' E with an altitude range of 1200-1700 m above sea level. The field and screen house experiment were carried out in the Regional College of Agriculture Bambili, North West Region Cameroon. It lies between latitudes 5 º and 6 ° North of the equator and longitudes 10 ° East of Greenwich Mean Time and at an altitude between 1600 to 2000 m above sea level.

2.2 Climate of the study site

This region is characterized mainly by two seasons, the rainy and dry season. The rainy season begins in mid March and ends in mid November while the rest season covers the rest of the year. The temperatures are usually cold within and average minimum and maximum temperature of 14 º C and 23 º C respectively. It has an average humidity of 70 % and 52 % in the rainy and dry

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season respectively. The annual rainfall is 2230 mm and rainfall is uniformly distributed from mid-March to mid-November (Camdem *et al*., 2020)

2.3 Field screening of potato cultivars in the field for late blight disease of potato

Four improved potato cultivars were used in this experiment (Pamina, Sassy, Desiree and Diaman) and two local varieties (Belo and Banso). Ten tubers of each potato variety were planted by putting one tuber per hole at 40 cm spacing between the plants on the 1st of April 2022 at the Regional College of Agriculture Bambili research farm. These potato varieties were replicated four times in randomized complete block design with three ridges per plot for each variety giving a total of 18 plots and 72 ridges. Each ridge measured 4 m length and 60 cm width with each plot separated by 2 m apart. Regular manual weeding of field plots was done at monthly interval after planting and molding was done six weeks after planting. Potatoes varieties were monitored for late blight disease symptom under disease pressure conditions. Disease incidence and severity were recorded immediately potato late blight appears.

2.3.1 Evaluation of disease incidence

Disease incidence was observed in the field at 42 days after planting (DAP). Data for disease incidence was collected on 6 plants in the middle ridges after 6 weeks of planting up to the 10th week at two week interval. The plants in the two other ridges and at the edges of the middle ridge were not evaluated because they served the role of pathogen invasion. Disease incidence was calculated using the formula:

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

2.3.2 Evaluation of disease severity:

Potatoes late blight severity was scored using a scale of 1-5; where 1= no symptom; 2= Low percentage (0-30 %) leaf infection 3= Moderate percentage (30-50 %) of plant leaves infected with late blight 4= High percentage (50-80 %) of plant leaves infected with late blight 5= High percentage (80-100 %) leading to complete damage of foliage. Following procedure adopted from Fukonang *et al. (*2000). Data was collected on 6 plants in the middle ridges from 42 DAP to 84 DAP at two weeks interval

2.4 Yield assessment of potato cultivars

Potato cultivars were assessed at 91 days after planting. The data collection was based on the number of tubers and tuber weight. The number of tubers in each plant was counted and recorded while tuber weight of each plant was measured in kg using an electronic scale.

2.5 Fungal collection, isolation and identification from infected plant materials

Leaves of potatoes showing young symptoms (lesions) of blight were collected early in the morning from the research field at Agricultural School Bambili. Each of the infected leaves were collected and placed inside two sheets of papers and transported to the Catholic University Laboratory Bamenda. These disease leaves were cut using a razor blade into small fragments of 2 mm from the advancing edges of the disease and placed on aluminum paper. The leaves fragments were surface sterilized in 5 % Sodium Hypochlorite (NaOH) for 10 seconds and rinsed

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in two successive changes of sterilized distilled water in Petri dishes. The leaves fragments were then dried on sterilized filter paper and three leaves fragments were placed in Petri dishes containing solidified V8 juice Agar culture medium containing Ampicillin (0.125g), Penicillin (0.125g) and Nystatin (0.01g) to inhibit bacterial growth. These dishes were labeled and placed inside a lamina flow chamber at room temperature of 20-23 ^{o} C. After four days of mycelia growth, mycelia were subculture two successful times to obtain a pure culture. Identification of *P. infestans* was by microscopic observation and confirmation by Nelson *et al*. (2011)

2.6 Preparation of spore suspension

Spore suspensions were prepared from 10 days old pure culture of *P. infestans* by flooding the Petri dishes with 5 ml of sterilized distilled water and the spores dislodged with a small brush .The spores were filtered using muslin cloth into a beaker. The spore concentration was determined with a heamocytometer and adjusted to 2.5×10^{4} spores / ml of distilled water. These spores were transferred into a syringe for inoculation in a screen house. (Manju *et al*., 2020)

2.7 Screen house experiment

The six (6) potato cultivars were planted in polythene bags filled with steam sterilized soils in a screen house. These plants were arranged in a randomized block design with three replicate and each replicate contained ten plants. These potato plants were inoculated 18 days after planting with spore suspension of *phytophthora infestans*. Inoculation was done on all the plants per replicate by using a syringe to inject the spore suspension on one spot on the plant leaves. The plants were watered daily throughout the growth period. Temperature and relative humidity were monitored using thermometer and Hobo meter respectively. A temperature range of 21 - 25^{o} C in the day and 18- 23^{o} C at night, and relative humidity range of 75- 85 % was maintained. Observations were carried out and blight lesion was measured as length and width of spread using a ruler and the area of lesion was calculated as Length x width. Data for lesion area was recorded at three days interval for 10 days (Manju *et al*., 2020).

2.7 Statistical analysis

Data for disease incidence, disease severity, yield and leaf area of late blight were subjected to analysis of variance (ANOVA) using statistical software (Originpro, 2021). The mean values were separated using Tukey's test and least significant difference (LSD) at $P\leq0.05$. Mean data was used to plot graphs for appropriate representation of the results.

3 RESULTS

3.1 Assessment of potato cultivars in the field for disease incidence of late blight

Disease symptom appeared on the leaves at 6 weeks after planting (WAP) as dark brown lesions in the field on some of the cultivars and gradually effected all the cultivars as days increases. No disease symptom was observed on these cultivars Desiree, Belo and Banso at 6 WAP. There was a significant difference ($P\leq0.05$) in mean **d**isease incidence at 6 and 8 WAP amongst the various cultivars. There was no significant difference ($P\leq0.05$) in mean disease incidence between cultivars at 10 WAP (fig.1). Pamina cultivar showed low mean disease incidence of 10 % at 6WAP under natural infection condition while all the potatoes cultivars had the highest mean

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disease incidence of 100 % at 10 WAP. Pamina, Sassy and diaman cultivars also had mean disease incidence of 100 % at 8 WAP.

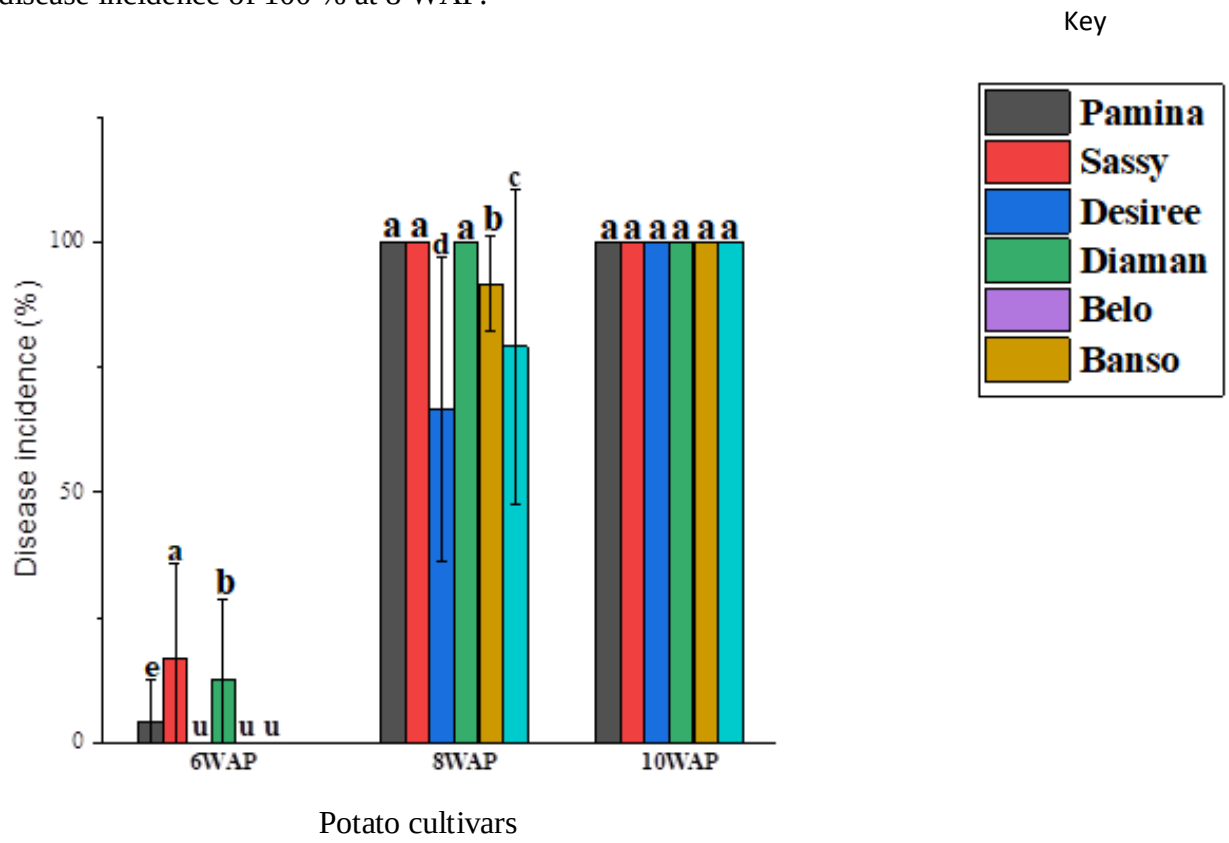

Fig 1: Disease incidence of late blight on potato cultivars at 6-10 weeks interval of infection

Bars represent mean disease incidence with standard errors

6 WAP = mean disease incidence of late blight at 6 weeks after planting, 8 WAP =mean disease incidence of late blight at 8 weeks after planting, 10 WAP = mean disease incidence of late blight at 10 weeks after planting

3.2 Disease severity of potato late blight disease

The rate of disease severity of PLBD on potato cultivars showed that there was an increase in disease with the age of the plants from 42-84 days after plant. Significant variation ($P \leq 0.05$) was observed in mean disease severity of PLBD with respect to the various cultivars at 6 to 12 WAP. Mean disease severity ranged from 1.0 % at 6 WAP to 5.0 % 12 WAP. The highest mean disease severity of PLBD of 5.0 % was recorded on cultivars, Pamina, Sassy and Diaman at 12 WAP. Belo and Banso cultivars had the lowest mean disease severity of PLBD of 1.0 % at 6 WAP (fig. 2). All the cultivars the improved potatoes cultivars (Pamina, Sassy, Desiree and Diaman) were highly susceptible to *P. infestans* compared to the local cultivars (Belo and Banso) that were susceptible.

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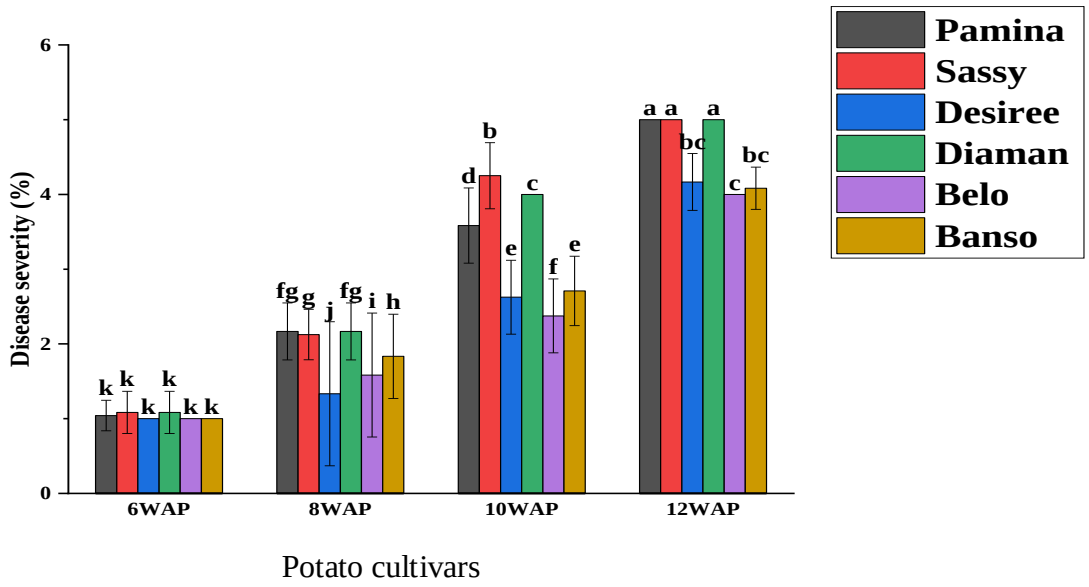

Fig 2: Disease severity of PLBD on potato cultivars at 6-12 weeks interval of infection

Bars represent mean disease severity with standard errors

6 WAP = mean disease severity of late blight at 6 weeks after planting, 8 WAP = mean disease severity of late blight at 8 weeks after planting, 10 WAP = mean disease severity of late blight at 10 weeks after planting, 12 WAP = mean disease severity of late blight at 12 weeks after planting.

3.3 Yield assessment of potato cultivars

The local cultivar Belo and improved cultivar Desiree had high mean numbers of tubers of 12.5 and 12 respectively. The tubers in these cultivars were smaller in size compared to the other cultivars that had larger sizes. Diaman cultivar had the lowest mean number of tubers of 6.0 and the tubers were moderate in size (fig 3). The largest sizes of tuber were observed in pamina cultivar. There was a significant differences ($P \leq 0.05$) in the mean number of tubers with respect to the various cultivars at 91 DAP. The mean number of tubers ranged from 6 to 11.5 in Diaman cultivar and Belo cultivar respectively.

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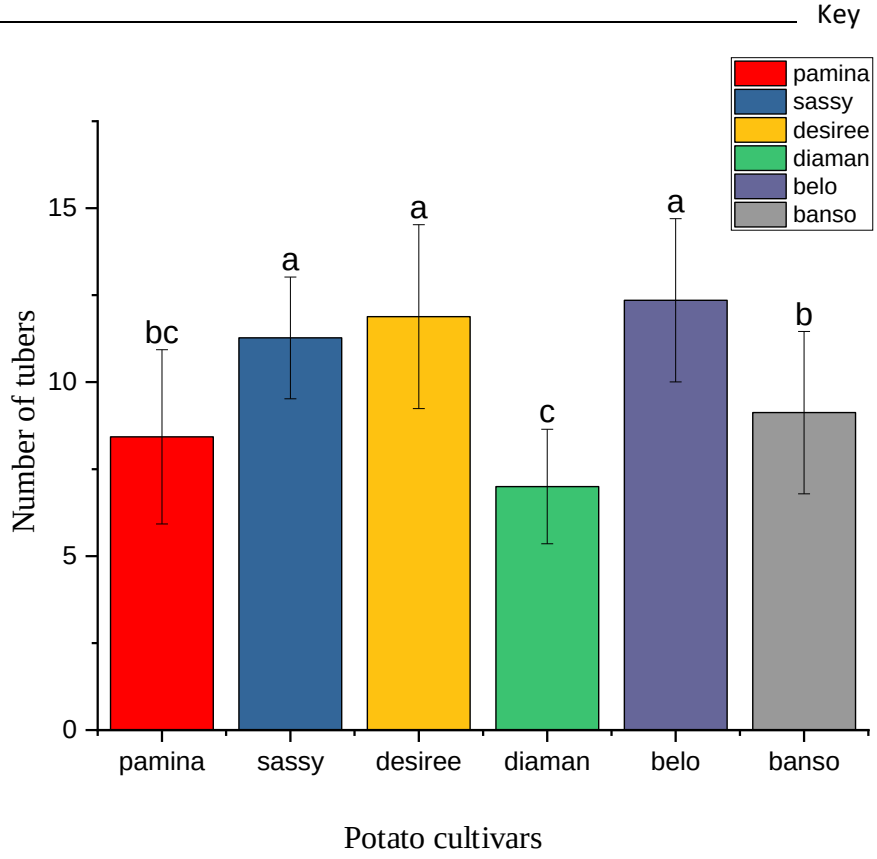

Fig3: Mean number of potatoes tubers at 91 DAP

Bars represent mean number of tubers with standard errors

91 DAP = mean number of tubers at 91 days after planting

3.4 Weigh of potato cultivars

There was a significant difference ($P \leq 0.05$) in mean weight of potatoes at 91 DAP among the cultivars. There was a mean maximum fresh storage tuber weight of 1.75 kg recorded on improved cultivar Pamina and 1.4 kg recorded in improved cultivar Sassy and mean minimum value of 1.02 kg recorded in local cultivar Banso. There was no significance ($P \leq 0.05$) difference between Desiree, Diaman and Banso (fig.4).

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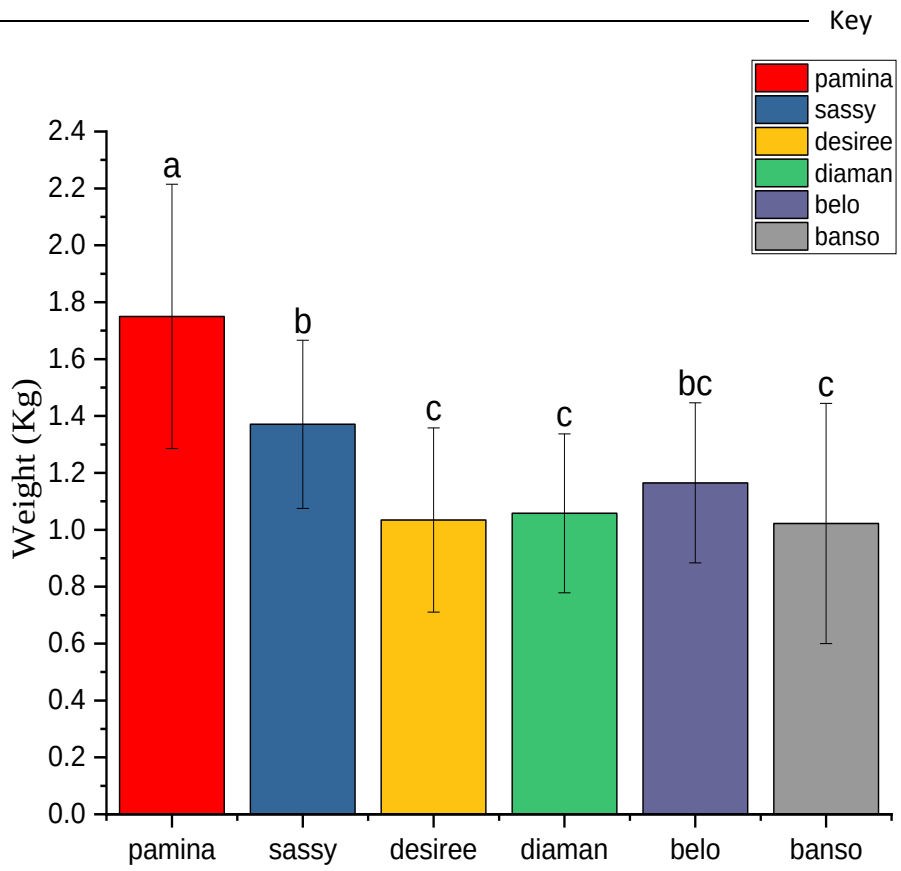

Fig4: Weight of potato tubers at 91 DAP

Bars represent mean tuber weight with standard errors

91 DAP = mean tuber weight at 91 days after planting

3.5 Test for virulence and pathogenicity of *phytophthora infestans* in the screen house.

Screen house test of cultivars was evaluated base on their reaction to fungi after inoculation. The variation in area of blight lesion amongst the different cultivars was significance ($P \leq 0.05$) with the highest mean lesion area of 27.2 m^2 of *P. infestans* growth at 12 days after inoculation recorded on Diaman cultivar followed by Sassy (14.45 m^2) and desiree. Belo cultivar recorded lowest mean blight lesion area of 0.04 m^2 at 12 day after inoculation and was rated as highly resistant (fig. 5). Blight lesion did not develop on Belo cultivar at 3 and 6 day after inoculation. The other cultivars were either resistant, intermediate or susceptible rang with blight lesion area ranging from 0.04m^2 to 27.2m^2.

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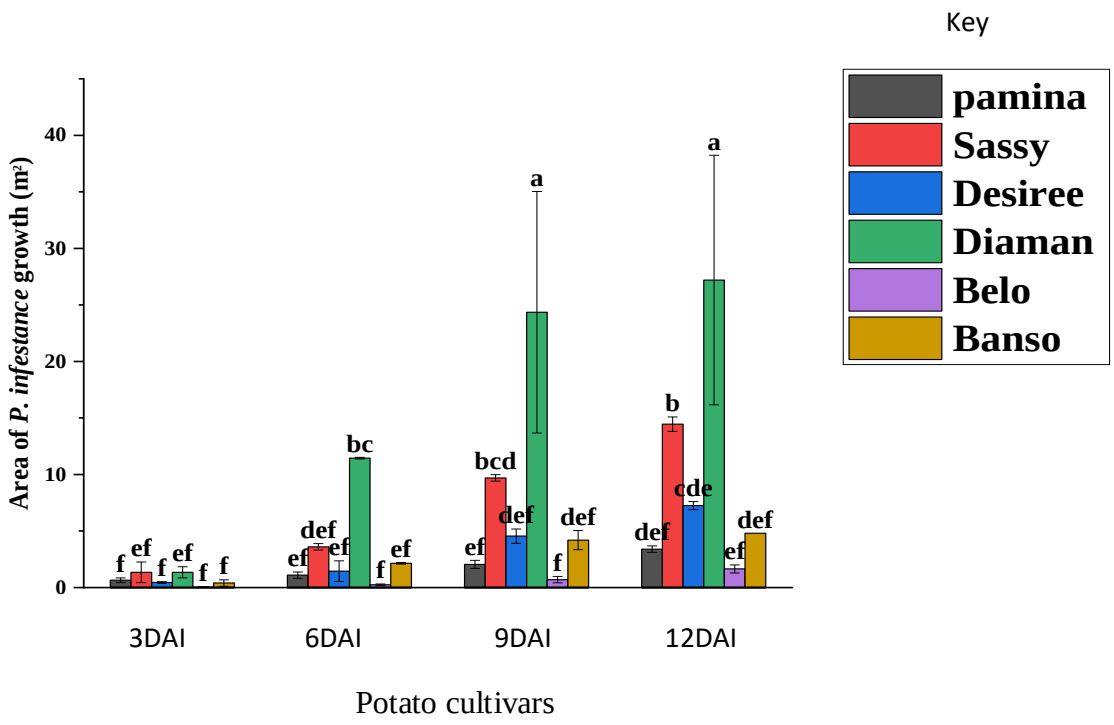

Fig 5: Area of *P. infestans* growth on potato cultivars at 3-12 DAI

Bars represent mean area of *P. infestans* growth with standard errors

3 DAI = mean area of *P. infestans* growth at 3 days after inoculation, 6 DAI = mean area of *P. infestans* growth at 6 days after inoculation, 9 DAI = mean area of *P. infestans* growth at 9 days after inoculation, 12 DAP = mean area of *P. infestans* growth at 12 days after inoculation.

4. DISCUSSION

Dark brown lesions were observed on the leaves on all the cultivars in the field at 6 weeks after planting which increases with the age of the cultivars. This is in accordance with report from Schumann and D'Arcy (2000) who state that dark brown lesions increase with age of the plants.

One hundred percentage of mean disease incidence was observed on all the improved and local cultivars of potato at 12 WAP. This could be due to high humidity, regular rains and favourable temperatures in the field that favoured disease development. Temperature of 10 to 25 ° C accompanied by heavy dew or rains promotes the spread of this disease (CIP, 1996). Also, Agrios (2005) who reported that on warm humid days the ripe sporangia detached and are dispersed by wind and rain.This sporangia caused infection on wet potato leaves and stems, they germinate and cause new infection of *Phytophthora infestans*. During frequent raining weathers, leaves of *Solanum tuberosum* landraces takes a shorter time to be destroyed by the disease than when rain falls at a normal rate. The damaging of the leaves in turn reduces the rate of photosynthesis resulting in a reduction in tuber yield. Improved Sassy cultivar gave the highest mean disease incidence of 100 % of PLBD at 8 WAP this indicated it was highly susceptible to

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PLBD compared to improved Derisee cultivar with mean disease incidence of 66.67 % of PLBD at 8 WAP, indicating that it is resistant to PLBD. This is in line with reports of Jaime *et al.*, (2014) who reported that potato genotypes which developed late blight symptom early are susceptible and genotypes that developed late blight symptoms late in the crop cycle are resistant.

Disease severity of PLBD on the improved and local cultivars of potato increases with age of plant at 6 to 10 WAP. Different potato cultivars showed different response to late blight (*Phytophthora infestans*) disease. The highest mean disease severity of PLBD of 4.25 % was seen on improved Sassy cultivar at 10 WAP. The increase in disease severity of PLBD with age of improved Sassy cultivar could be due to the inability of the cultivar to resist the pathogen and prevailing environmental conditions such as increase in humidity and favourable temperatures. Mukalazi *et al*. (2001) reported that a reduction in disease severity in moderately resistant varieties was due to their ability of minimizing changes in the population structure of *P. infestans.* Also, Deadman (2006) reported that the resistance to late blight disease may be affected by environmental conditions**.** The most effective approach to combat plant disease is the use of host resistance (Subedi, 2015). The rate of disease severity was less in local cultivars (Belo, Banso) and improved cultivar Desiree compared to other cultivars indicating they were susceptible late blight disease. Sharma (2013) indicated that late blight severity observations in the field started at 30 days after planting and continued up to 76 days until the susceptible cultivars had 100 % infection. It was observed that late blight directly affects the yield attributing characters like vine mass and tuber yield.

The local cultivar Belo and Diseree had high mean numbers of tubers of 12.5 compared to other cultivars. The tubers in these cultivars were smaller in size compared to the other cultivars that had larger sizes. Improved cultivar Pamina recorded the highest mean tuber weight of 1.7 kg and had larger tubers compared to the other cultivars which were responsible for the large weight. Belo and Desiree cultivars were susceptible to late blight disease so many tubers were produced while Pamina was highly susceptible thus few tubers. **There is higher yield of tubers observed under low disease severity and lower yield of tuber in higher disease severity (Shrestha *et al*., 2019).**

The highest mean lesion area of 27.2 m^2 of *P. infestans* growth at 12 days after inoculation was recorded on Diaman cultivar followed by Sassy (14.45 m^2) and desiree. The differences in the mean area of *P. infestans* among the cultivars could be due to variations in their resistance and susceptibility to the pathogen. This study is in line with Pittis *et al*. (1994), who reported that oospore of *P. infestans* was formed in leaves of potato cultivars (Bintje) 10 days after inoculation with a mixture of equal amount of sporangiospores. Also, abundant oospore production in moderately resistant potato cultivars can be explained by the fact that leaf of these potato cultivars showed delayed deterioration resulting in extra time for production of oospores. The *invitro* screening technique for resistance to potato late blight disease thus demonstrate a good avenue for studying host pathogen, relationship and plant disease resistance. it also provide an alternative reliable, rapid and simple method for evaluating samples of potatoes cultivars for resistance to potato late blight disease in potato breeding programmes.

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5. CONCLUSION

The highest mean disease severity of (PLBD) of 5.0 %, least numbers of potatoes tuber and highest mean lesion area of (PLBD) was recorded on improved cultivars (Pamina, Sassy, Diaman), indicating that they are highly susceptible to potatoes late blight disease compared to the local cultivars (Belo, Banso) and improved cultivar (Desiree) that were susceptible to potatoes late disease and produced average yields. These local cultivars and improved cultivar Desiree, could be planted in the field by farmer without using chemicals to control the field.

Potato evaluation for potato late blight disease and other microbial is particularly important in low-input agricultural systems where microbial, insects and weed pests are major problems. Given the obvious needs in terms of cost and environmental impact, to reduce the pesticide use, the selection and production of resistant potato cultivars to pest and disease, is a major strategy towards meeting international challenges in food security, poverty alleviation, agricultural productivity and environment conservation.

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author MEB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CBL, ART and NDK managed the study design, statistics and the literature searches. All authors read and approved the final manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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