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Pathogenicity and Virulence of Alternaria Blight Isolates on Three Selected Cultivars of Sweetpotato in Uganda

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Abstract: Alternaria species cause diseases to more than 380 host plants with the damage causing 60%-100% plant death. This makes it a species of interest for sweetpotato researchers and farmers. However, there is a shortage of information since limited research has been conducted on Alternaria species that causes Alternaria blight of sweetpotato. This study aimed to fill this research gap. A study was carried out on the Alternaria blight disease of sweetpotato. *Alternaria bataticola* and *Alternaria alternata* were isolated from the diseased sweetpotato stems, vines, and leaves. From the cultures, Alternaria bataticola was the most frequently isolated species from the infected plants, glaringly higher in frequency than *Alternaria Alternaria*. Specifically, pathogenicity tests were conducted on six representative isolates tested on three selected varieties in the study. The pathogenicity tests on the susceptible sweetpotato variety using all six fungal isolates showed that only *Alternaria bataticola* was more aggressive producing symptoms of larger lesions that are dark grey in color, it produces characteristic symptoms of black lesions on the leaf abaxial, nodes, and on the stems. The observation of the characteristic symptom of dark grey lesions with concentric rings on the leaves and re-isolation of the pathogen from infected leaves and stems confirmed that both *Alternaria bataticola* and *Alternaria alternata* were responsible for the sweetpotato Alternaria blight disease. Isolates, cultivar (isolate × cultivar) interaction, and experimental effects were obtained.

Key words: Pathogenicity, isolate, *Alternaria alternata*, *Alternaria bataticola*, cultivar, virulence.

1. Introduction

Sweetpotato is consumed by large numbers of rural families, but its production is variably affected by several biotic and abiotic factors that include: bacterial, fungal, and viral diseases and nematodes [1, 2]. The levels of damage due to diseases and pests are due to many factors like variety, virulence of the pathogen, and genotype by environment interaction.

Alternaria blight disease is one of the sweetpotato fungal diseases that cause leaf, stem and petiole blight in sweetpotato. However, both *A. alternata* and *A. bataticola* are the two Alternaria species that have been isolated and identified from sweetpotato plant tissues. *A. bataticola* has been reported as a more prevalent and aggressive species than *A. alternata* [2, 3]. Most work on impact through field evaluation has been carried out in Uganda where the disease is especially serious. Yield loss depends on variety, region and cropping season. All commonly grown and preferred varieties are susceptible. The disease is most serious in crops at mid and high elevations, those in the cool, moist southwestern highlands (altitude above 1,500 masl and annual rainfall 900-1,350 mm), and in parts of the central Lake Crescent Region, but less so in the drier regions of eastern and northern Uganda [4]. Severe losses in many parts of the world have been attributed to high levels of

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incidence and severity of this disease due to conducive environments for the proliferation of the pathogen. In places where conditions favour the disease, losses of storage root yield of 50%-90% are reported, especially where Alternaria blight and sweet potato virus disease occur together [5]. Such is the importance of these diseases that makes it a species of interest for sweetpotato researchers and farmers and thus the focus of breeding. Development of resistant varieties is the most appropriate approach to control the disease and the concept is now to evaluate potential parents for stability in the expression of Alternaria blight resistance. Natural infection does not offer sufficient inoculum pressure difference between resistant and susceptible genotypes. There is need to inoculate varieties under controlled conditions (screenhouse) in order to establish adequate disease pressure. This would enable to evaluate disease severity and calculation of disease incidence [6].

Alternative hosts and volunteer crops provide sources of primary inoculum and planting material infection has been reported as sources for Alternaria blight transmission [7]. In the host crop, the secondary spread of Alternaria blight is mainly through rain-splashed spores, and wind-borne dispersal of dry conidial masses [8].

Subsequently, the pathogen invades the adjoining leaves and gradually progresses on the plant tips. The lesions join and form enlarged elongated black lesions on stems, leaves, and nodes. While many researchers in different parts of the world have done extensive work on Alternaria blight on other crops, no quantifiable information is readily available on sweetpotato, additionally, very limited information is available on the pathogenicity of the Alternaria fungi in Uganda. This study was, therefore, geared to identify the fungus causing Alternaria blight disease on sweetpotato and to confirm its pathogenicity on the host. This research work aims to study the Alternaria blight disease of sweetpotato with the following objectives:

(1) To isolate and identify organism(s) responsible for Alternaria blight disease in sweetpotato;

(2) To determine the pathogenicity of these organism(s).

2. Materials and Methods

2.1 Experimental Sites

The laboratory experiments were conducted in the Biotechnology Laboratory, the Department of Crop Science at Makerere University.

Two experiments: One laboratory and one screenhouse were carried out at Makerere University Agricultural Research Institute Kabanyolo (MUARIK) located 19 km north of Kampala at an Altitude of 0028° N, longitude of 32.37° N and at a mean altitude of 1,200 m above sea level (asl). It receives an annual rainfall of 1,300 mm with a mean maximum and minimum temperature of 28.5 °C and 13.0 °C respectively. The area has ferallitic heavy but well-drained soils.

2.2 Sample Collection and Isolation of Alternaria Pathogen

Symptomatic sweetpotato plant tissues (leaves, vines and petioles) were collected from 17 sweetpotato growing districts of Uganda. These locations include: Iganga, Kamuli, Busia, Sironko, Soroti, Apac, Masindi, Hoima, Kibale, Luwero, Wakiso, Mpigi, Masaka Rakai, Mbarara, Ntungamo and Kabale.

Diseased samples with clear Alternaria lesions, were collected and placed in paper bags to avoid desiccation and cross contaminations, kept in an ice box, and transported to the laboratory.

2.3 Isolation and Identification of the Pathogens Associated with Diseased Sweetpotato

Procedures for Isolation of the Pathogen

To obtain isolates from sweetpotato plant tissues, 5-10 g of plant materials with lesions were cut and put in 500 mL of tap water then rinsed in 500 mL sterile water and agitated by hand shaking for 30 min. The stem pieces were surface sterilized using 3% sodium hypochlorite (NaOCl) for 5 min and rinsed in 3 changes of sterile distilled water. Excess water was drained

using sterile tissue paper. The surface sterilized infected sweetpotato vine and leaf samples were put in a moist chamber and incubated for 24 h to allow sporulation as described by [9].

2.4 Preparation of Potato Dextrose Agar (PDA)

Thirty-nine grams (39 g) of PDA was suspended in 1,000 mL of cold distilled water in a conical flask. The conical flask was closed with a tight cotton plug and heated to boiling to dissolve the medium completely. It was then sterilized by autoclaving at a set temperature of 121 °C for 15 min and allowed to cool to 45 °C before being dispensed in sterile Petri dishes. The media was allowed to solidify before the use. The procedure was adopted from [10]

2.5 Single Spore Isolation

A PDA plate method used by Narayanin *et al.* [11] was adopted where a total of 40 putative single spores of Alternaria colonies were randomly picked from the lesions on infected plant parts under a binocular microscope and seeded into the surface of PDA using a tip of a sharp, sterile inoculating needle. Inoculated plates were incubated on a laboratory bench at room temperature (20-24 °C). Conidial germination on the plates was checked daily, and upon germination, agar blocks bearing single germinated conidia were cut aseptically seeded into fresh, sterilized media. Plates were incubated for 14 days at room temperature and natural lighting conditions (20-24 °C and 12 h light). Plates of primary media were centrally inoculated with 2 mm diameter plugs taken from the edge of actively growing 4-day-old cultures and then incubated at 25 °C for 72 hrs.

2.6 Identification of the Pathogens

Comparison of cultures/isolates with known culture structures of *Alternaria bataticola* (*A.b*) and *Alternaria alternata* (*A.a*) was done using a descriptor used by Woudenberg *et al.* [12] to identify Alternaria isolates from sweetpotato. This technique aided the identification and selection of representative isolates for inoculation. Molecular characterization was done using Restriction Fragment Length Polymorphism (RFLP) and Random Amplified Polymorphic DNAs (RAPDs). Molecular technique was used to accurately distinguish isolates at a genetic level, because morphological similarity alone may not be conclusive in providing the differences in the isolates, that may be similar phenotypically but not necessarily be similar genotypically. This had to be done to enable the selection of representative isolates for pathogenicity tests.

2.7 Screenhouse Experiment

The experiment was in a Randomized Complete Block Design (RCBD). Eight-week-old plants of varieties; Ebwanaterak, Magabari, and NASPOT 1 were inoculated with six representative Alternaria isolates with inoculum concentration standardized to approximately 400-600 conidia per milliliter with a hemocytometer.

To study the variability in pathogenicity and virulence of Alternaria species causing Alternaria blight disease in sweetpotato in Uganda, plants originating from micropropagation were transplanted in well-drained sterilized soils in 30-cm diameter plastic buckets. Plants were used for inoculation at the 8-week-old stage, using six representative isolates; three for each species (*Alternaria bataticola* and *Alternaria alternata*) respectively. Three of the cultivars used: Ebwanaterak, Magabali, and NASPOT 1 represented a range of resistance, moderate, and susceptibility to Alternaria blight respectively.

The procedures adopted are stated below.

2.8 Inoculum Preparation

Conidial suspensions were prepared from 14-day-old monosporic cultures grown on PDA media and Vine decoction media. The plates were flooded with sterile distilled water (10 mL per plate). Conidia was dislodged by gently scraping the surface of the media using sterile glass rods, and the suspension produced was strained

through two layers of sterile cheesecloth. Conidial concentration was determined using a hemocytometer and standardized at 5.4-6.0 × 10^6 per conidia/mL.

2.9 Inoculation and Incubation of Test Plants

Six selected sweetpotato Alternaria isolates representing a range of morphological types and Alternaria species were tested for pathogenicity to sweetpotato leaves and vines. The plants were inoculated by spraying 500 mL of inoculum to run off on both sides of the 10 leaves on each plant using a hand sprayer.

The inoculation was repeated until consistent results were obtained. Control plants were sprayed with sterile distilled water. Disease symptoms on potted plants were scored after 5 to 7 days of inoculation. Lesion size was measured. To minimize the environmental effect, inoculations were performed within the same period. Severity scores were recorded weekly for four weeks using the rating below: 1 = no lesions, 2 ≤ 1 mm in diameter, 3 = lesions 1-5 mm in diameter, and 4 lesions > 5 mm in diameter, while close attention was paid to older leaves since there are hints in the literature that older leaves are first affected before the younger leaves [13].

2.10 Laboratory Bioassay

The experiment was factorial in Randomized Complete Block Design (FRCBD).

Detached leaves from eight-week-old plant varieties; Ebwanaterak, Magabari, and NASPOT 1 were inoculated with a syringe using six representative sweetpotato Alternaria isolates. For each isolate tested, three fully grown expanded leaves were placed in squared plastic dish containers, aligned with a sterilized moist sponge, and on tissue paper to avoid leaf desiccation. The tests were conducted on wounded leaf midrib and leaves. To inoculate the leaves, 20 µL of a conidial suspension was placed on each leaf and targeting the wound. Control leaves of each variety were sprayed with sterile distilled water. The plastic containers were covered with lids to maintain high humidity and were incubated at 20 °C for 7 days. After incubation resulting lesions (as evidenced by the diameter of the lesions produced following inoculation) were recorded daily for 7 days and scored on a point rating system of 1 = no lesions, 2 ≤ 1mm in diameter, 3 = lesions 1-5mm in diameter, and 4 lesions > 5 mm in diameter.

All data obtained were subjected to analysis of variance (ANOVA) in Area Under Disease Progressive Curve (AUDPC) at 5% probability levels of the *F*-test. Significant differences in treatment effects were identified using Duncan's multiple range test and the least significant differences test at a 5% probability level.

2.11 Isolation and Culturing

Seven days after inoculation, leaves and vines showing symptoms were detached and re-isolated to fulfill Koch's postulates by plating them with PDA media.

3. Pathogenicity Test Results

All inoculations were successful with a high percentage of conidial germination (70%-95%) obtained with each inoculation. Both species of Alternaria blight isolates (*Alternaria bataticola* and *Alternaria alternata*) (Table 1) infected the three varieties in Table 2 within 5-10 days in the isolate virulence pathogenicity determination experiment.

ANOVA for Alternaria blight for Area under disease progress curve (AUDPC) data showed that the genotype, isolate and isolate × Genotype were all significantly ($p < 0.05$) different for the AUDPC (Table 3, 4). The resistant genotype (Ebwanaterak) exhibited low AUDPC levels compared to the most susceptible genotype (NASPOT 1) which had a high AUDPC.

The AUDPC values for the resistant genotype (Ebwanaterak) were lower than those of the moderate and susceptible varieties, Magabari and NASPOT 1 respectively. The susceptible variety, (NASPOT 1) had

severe disease symptoms as compared to the resistant variety (Ebwanateterak) which had very mild symptoms. However, both screenhouse and laboratory bio-assay experiments were not different in terms of significance, all 6 isolates were able to display disease symptoms in a susceptible variety (NASPOT 1) in only 5 days following inoculation, whereas Magabari, a moderate cultivar, and Ebwanaterak showed symptoms in 10 days after inoculation for isolates MPG 259 (4), KML 286 (5), and NTG 215 (6) (Fig 1), and in two weeks for isolates BSA 27 (1), HMA 137 (2), MSK 141 (3) (Fig 2) respectively. Control plants remained healthy for the entire period of evaluation Disease symptoms were more evident in a susceptible variety (NASPOT 1) as numerous brown spots which progress to black spots, coalesced to form black lesions as a result of many spores. The leaf is seen to wilt resulting in the yellowing of colour before dropping off from the plant forming a carpet under plants (Fig 3). Disease symptoms were observed on leaves' petioles and stems/vines. On wounded leaves of the bioassay experiment, the symptoms started as a spot and enlarged to big lesions, killing the entire leaf (Fig. 4). The symptoms extended from the lower older leaves to the younger leaves.

Disease symptoms occurrence in an infected plant can be compared with a healthy control plant and this criterion can be expressed as a score on a point rating system scale. Symptoms like lesion size and appearance, severity was evaluated using a scale on a point rating system. Considering the scale used the tested sweetpotato varieties' reaction to these isolates was expressed as Resistant (R) = resistant with a score of 1-2, Moderately resistant (MR) with a score of 2.1-3, and highly susceptible with a score of 3.1-5, these were Ebwanaterak, Magabari and NASPOT 1 respectively.

Table 1 List of representative isolates used in pathogenicity and virulence tests for group 1 (*Alternaria alternata*) and 2 (*Alternaria bataticola*).

Code	Isolate name	District of origin	Alternaria species
Group 1: *Alternaria alternata*			
1	BSA 27	Busia	*Alternaria alternata* (*A.a*)
2	HMA 137	Hoima	*Alternaria alternata* (*A.a*)
3	MSK 141	Masaka	*Alternaria alternata* (*A.a*)
Group 2: *Alternaria bataticola*			
4	MPG 259	Mpigi	*Alternaria bataticola (A.b)*
5	KML 286	Kamuli	*Alternaria bataticola* (*A.b*)
6	NTG 215	Ntungamo	*Alternaria bataticola* (*A.b*)

Table 2 List of representative cultivars used in pathogenicity and virulence tests.

Code	Accession name	Cultivar name	District of origin	Alternaria status	Status
1	PAL 1303	Ebwanaterak	Pallisa	Resistant	Landrace
2	KBL 618	Magabari	Kabale	Moderate	Landrace
3	NIS/91/52	NASPOT 1	Breeding line	Susceptible	Released

Table 3 Analysis of variance (ANOVA) of the area under disease progress curve (AUDPC) for different sweetpotato varieties in the screenhouse.

Source of variation	df	SS	MS	Vr	Fpr
Isolate	5	2,564.753	512.951**	74.15	0.001
Variety	2	4,475.59	2,237.795**	323.5	0.001
Isolate × variety	10	157.626	15.763^{ns}	2.28	0.012
Residual	1,355	9,373.284	6.918		
Total	1,379	16,923.638			

** Significant at ($p < 0.05$), ns = non-significant.

Table 4 AUDPC ANOVA means on three cultivars' response to inoculation with six Alternaria isolates.

Isolate	Species	Origin	Cultivar disease response				Isolate/cultivar
			Ebwanaterak	Magabali	NASPOT 1	Mean	Interaction
BSA27	*A. alternata*	Busia	8.268	9.606	13.612	10.495	1
HMA137	*A. alternata*	Hoima	9.162	10.774	13.549	11.152	1
MSK141	*A. alternata*	Masaka	9.818	11.281	14.666	11.933	1
MPG259	*A. bataticola*	Mpigi	11.231	12.231	15.656	13.226	2
KML286	*A. bataticola*	Kamuli	12.158	12.766	15.48	13.468	2
NTG215	*A. bataticola*	Ntungamo	13.016	14.059	16.216	14.430	2

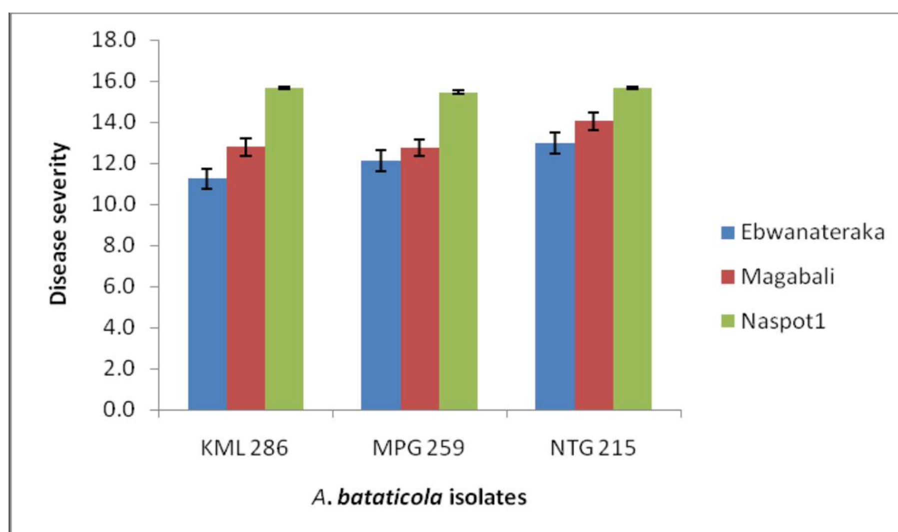

Fig. 1 Severity of *Alternaria bataticola* on three sweetpotato varieties.

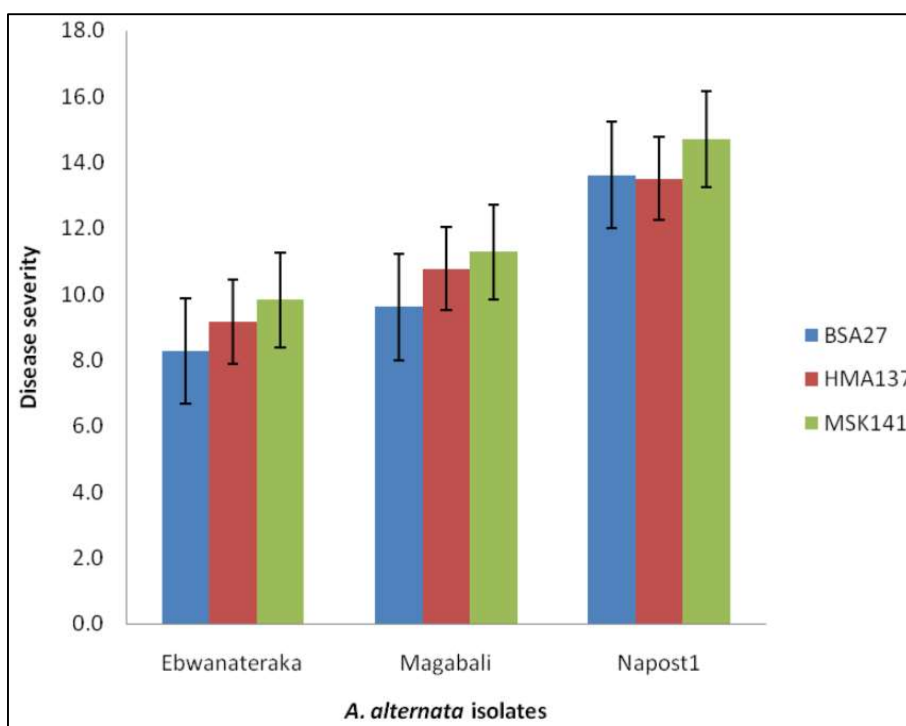

Fig. 2 Severity of *Alternaria alternata* on three sweetpotato varieties.

Fig. 3 Symptoms of *Alternaria bataticola* on a susceptible cultivar (NASPOT 1) in screenhouse experiments.

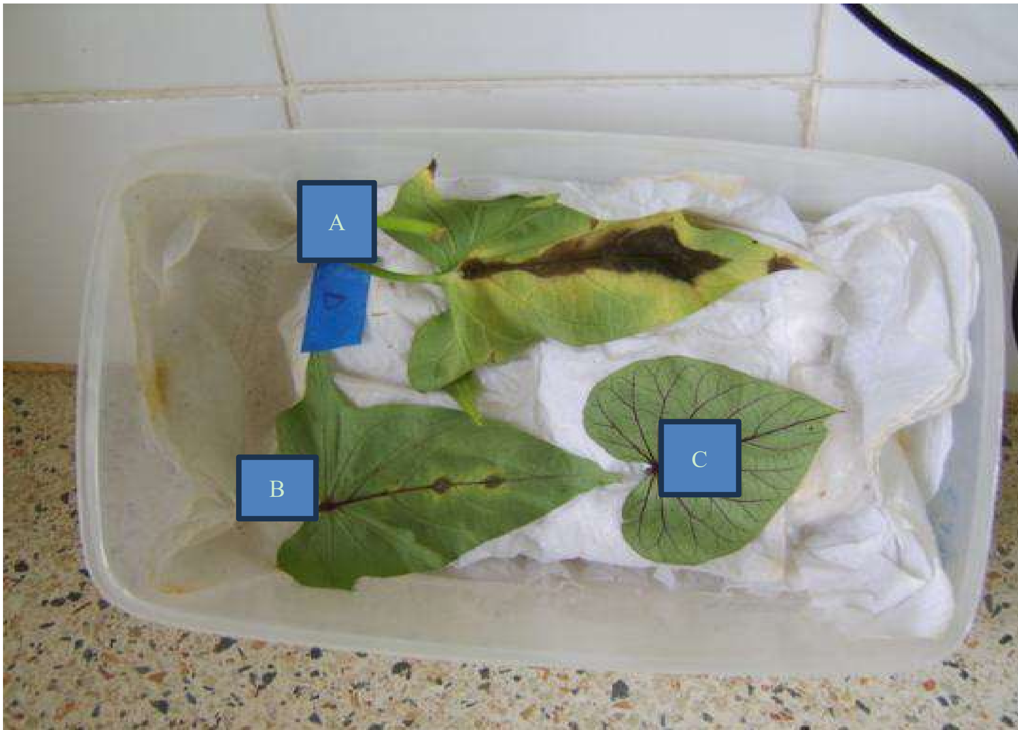

Fig. 4 Symptoms of *Alternaria bataticola* in bioassay experiment. A = NASPOT 1 (Susceptible); B = Magabali (Moderately) susceptible and C = Ebwanaterak (Resistant).

4. Discussion

In the present study, we noted that all two species of Alternaria (*A. bataticola* and *A. alternata*) were identified, and they both cause disease symptoms in sweetpotato and they seem to form a synergetic role in disease development. The severity of Alternaria blight was like for any other fungal disease, varies with species, variety, asexual growth stages, and latent period. The diversity can be attributed to the existence of unknown sexual stages [14]. This asexual variation can be a result of different types of pathotypes that keep on emerging and mutating from the existing ones. The study indicated *A. bataticola* as the most aggressive and virulent species in terms of mycelial growth and pathogenicity test compared to *A. alternata*.

All *A. bataticola* isolates caused clear symptoms in all the varieties. This is consistent with previous reports [15], but within the species, the isolates of the same species responded differently under the same screen house conditions depending on the source although, in terms of mycelial growth, and color including the spore shape they were very similar. This explains that even when all other features are similar, there may be differences within the species that may be due to mutation and developing pathotypes.

Cultivar NASPOT 1 was very susceptible to all isolates, while cultivar Magabali varied from susceptible to intermediate according to the isolate type used. However, the cultivar Ebwanaterak was either intermediate or resistant. It was also noted that isolate type affected the virulence; specifically, isolates of *Alternaria bataticola* (KML 286, MPG 259, and NTG 215) differed significantly in virulence from the *Alternaria alternata* isolates (BSA 27, HMA 137, and MSK 141) that caused only mild symptoms with a significant level of $p < 0.005\%$. In this experiment, the *A. bataticola* isolates displayed aggressiveness both during inoculation and in culture growth.

Results of the combined ANOVA of AUDPC on disease severity for all varieties used in the experiment for studying the effects of isolate, cultivar (isolate × Cultivar) revealed that isolate and cultivar interaction was significant ($p \leq 0.05$).

In the bioassay experiment, the results were not different from the screen house, the isolates gave similar results with *A. bataticola* isolates (KML 286, MPG 259, and NTG 215) having the most severe symptoms on cultivar NASPOT 1 (A) (susceptible), mild symptoms on cultivar Magabari (B) and extremely mild symptoms on cultivar Ebwanaterak (C) (resistant) (Fig. 4). In the re-isolated samples from the symptomatic plants that were inoculated in the screenhouse, we proved that Alternaria isolates were the causal agent for Alternaria blight disease in sweetpotato because they were able to fulfill Koch's postulates. After all, the symptomatic plants reproduced the same fungi of Alternaria that were causing symptoms in sweetpotato fields.

However, all the tested isolates shared a lack of host specificity since most of them infected sweetpotato and caused disease symptoms. Equally important to note is that, the severity of Alternaria depends upon weather conditions prevailing, varieties, age of host plants, and virulence of the pathogen are explained [16].

The difference in the cultivar reactions to inoculation by displaying varied symptoms was of interest in this study and perhaps it will be interesting to elucidate further the key pathogenic determinants that are specifically involved in triggering disease symptoms. In this study, inoculum source, alternative host, and cultivar have been reported [17] as the determinants for the development of brown spot and black pit of potatoes. Similarly, a temperature range of 20-25 °C and a duration of wetness duration of 48 hrs as the ideal conditions for the spread of Alternaria blight infection [18]. However, the importance of all the mentioned factors (wetness period, inoculum, age, and variety) does not exclude the fact that some genotypes like NASPOT 1 are inherently more susceptible and easily succumb to the disease at the early stages of its growth as long as the weather is conducive for the pathogen to thrive.

This finding is in agreement with [19] where landraces are described as having good traits that can be utilized in breeding for improved cultivars. Overall, the study identified the importance of Alternaria blight in sweetpotato production and the existence of promising varieties to resist the risk of Alternaria blight. Meanwhile, future research should focus on the evaluation of promising varieties and the integration of management disease management practices

However, many scholars have in their studies identified tolerant landraces to Alternaria blight which can be used in future breeding programs to develop tolerant varieties against Alternaria blight [20]. However, the resistance levels exhibited by different cultivars do not only remain on their level of resistance or susceptibility but show why other researchers venture into a mindset and venture into the plant physiology aspect that explains plant-pathogen interaction. Taj *et al.*'s [21] findings reported that the effect of host-specific Alternaria toxins at physiological, and biochemical promotes an understanding of pathogenesis, and may cause a complex of symptoms affecting individual crops. The low and high level of tolerance to disease is attributed to the differences and changes in plant biochemical composition responsible for defense mechanisms [22]. Therefore, understanding the mechanism plants employ to defend themselves against pathogens may lead to novel strategies to enhance disease resistance in crop plants [23].

Further studies in this direction may provide information regarding host-pathogen interaction which can be utilized for resistance breeding to develop desirable traits by incorporating resistance in market-preferred but susceptible sweetpotato genotypes.

According to [24], in their work on brassica, with greater awareness of variation in the tolerance and of cultivars to pathogen attack, it is advised that it should be possible to make more accurate correlations between the severity of Alternaria blight and the phenolic compounds, which could also be applied for the case of Alternaria blight of sweetpotato. Nevertheless, improved phytosanitary measures: quarantine, sanitation, use of disease-free vegetative propagules for all new plantings, and rouging of disease plants from within plantings offer considerable benefits for controlling diseases, in other words, it requires integrated disease management [25, 26]. Another option is to focus on genotypes that carry resistance-related genes, given that resistance to Alternaria blight is polygenic [27].

5. Conclusion

The conclusion drawn from the study of the three tested varieties indicates that the resistant variety exhibited mild reactions to the disease, whereas the moderately resistant variety (Magabari) and the susceptible (NASPOT 1) varieties all showed symptoms. None of the genotypes evaluated under screenhouse conditions appeared immune. Overall, the study identified the importance of Alternaria blight disease in Uganda and the existence of promising varieties to resist the risk of Alternaria blight. These results show that the cultivar Ebwanaterak, a landrace, is resistant to Alternaria blight species so it can be used as a source of resistance in breeding for resistance to Altrenaria blight and it should also be recommended for farmers to take it up. Additionally, the most aggressive isolates identified of *Alternaria bataticola* should be used to screen sweetpotato populations for Alternaria blight resistance.

The development of resistant varieties is the most appropriate approach to control the disease and the concept is now developing to explore the built-in plant defense mechanism to pathogen attack. Breeding for disease resistance has become a prime concern for breeders over the years. To address this challenge, sweetpotato breeding programs have to focus on crossing resistant/tolerant and susceptible varieties, enabling the transfer of quantitative resistance but to speed up this breeding process, it is paramount that a broader approach is investigated, which includes understanding the biochemical components of the plant.

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