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Variability in Susceptibility of Pea Varieties to *Ascochyta* Blight Disease and Its Implications for Control in Molo Sub-county, Nakuru County, Kenya.

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Abstract

This study aimed to assess the susceptibility of different pea varieties (Grano, Kienyeji, Kigondoro, and Kiritho) to *Ascochyta* spp., a significant threat to pea (*Pisum sativum*) production. Disease severity scores and lesion radii data were analyzed using ANOVA and the least significant difference (LSD) test was used to separate the means. *Ascochyta* blight symptoms including purple, black, or brown spots and lesions were typical on both leaves and pods. Other symptoms observed included lower leaf blight and sunken lesions on pods. *Ascochyta* spp. pathogen was subsequently isolated from the diseased plant and identified based on the colony morphological characteristics. After being grown on potato dextrose agar (PDA) under an incubation temperature of 28°C, the fungal colony exhibited a typical grey colour and a woolly texture with irregularly defined edges. The study in Molo sub-county, Nakuru county, revealed significant variability in disease susceptibility among the examined pea varieties. The one-way ANOVA analysis had a p-value of 0.079, failing to reject the null hypothesis, indicating no significant difference in resistance to *Ascochyta* blight disease. However, Fischer's Least Significant Difference (LSD) test showcased significant differences in susceptibility among the Grano pea variety compared to the Kienyeji ($p = 0.030$), Kigondoro ($p = 0.030$), and Kiritho ($p = 0.043$) pea varieties. These findings are important to farmers in selecting varieties that have some level of resistance to *ascochyta* blight disease affecting garden pea. In the long run, this study helps in enhancing the sustainability of garden pea cultivation in Kenya. This study also adds knowledge into the existing integrated disease management (IDM) strategies for the management of *ascochyta* blight disease in Garden pea. This research primarily addressed disease severity and not crop yield and quality and other related factors that are also of important concern as well; hence, other researchers may consider investigating these other factors. Besides, this study helps with increasing the agricultural productivity and sustainability, in which the management of crops involves disease-resistant variety selection.

Key words: *Pisum sativum*, Varieties, *Ascochyta* spp. ANOVA, LSD, PDA, severity, susceptibility, IDM.

Abbreviations

AB	Ascochyta Blight
ANOVA	Analysis of Variance
BCA	Bio Control Agent
CFUs	Colony Forming Units
DSI	Disease Severity Index
FAO	Food and Agriculture Organization
IDM	Integrated Disease Management
LSD	Least Significant Difference
MDGs	Millennium Development Goals
PDA	Potato Dextrose Agar
SPSS	Statistical Package for the Social Sciences
SDGs	Sustainable Development Goals

1.Introduction

Garden pea (*Pisum sativum*) belongs to the family Fabaceae. It plays a pivotal role in sustainable food production, human and animal health, global food security, and the resilience of agricultural systems (Smykal et al., 2017; Alam et al., 2020). Garden pea contains 18-30 % protein, 35-50% starch, and 4-7 % fiber and is consumed fresh and dry (Wu et al., 2023). As a result of its high protein content, it is an alternative source of protein to animal-based proteins (Ibrahim et al., 2020). Additionally, it has a critical role in crop rotation due to its ability to fix nitrogen in the soil (Mirzad et al., 2023). This is due to the symbiotic relationship that the plant has with the root nodule bacteria in the soil, which enables it to fix over 70% of atmospheric nitrogen (Sinjushin et al., 2022). As a result, the demand for synthetic nitrogenous fertilizers is reduced, creating a more sustainable farming ecosystem and increasing biodiversity (Ibrahim et al., 2020; Sinjushin et al., 2022).

Despite the benefits that *P. sativum* presents in the local and international market, its productivity is hindered by biotic and abiotic factors. For instance, water stress, extreme temperature stress, pests, and diseases are limiting factors in obtaining maximum yield (Ibrahim et al., 2020). Ascochyta blight is one of the most notorious diseases affecting garden pea production worldwide. A complex of fungi causes the disease, with incidence and severity rates of 62.4% and 64.6%, respectively (Riccioni et al., 2019).

Many experiments indicate a significant variation in the susceptibility of *P. sativum* varieties to different pests and diseases (Assen, 2020; Joshi et al., 2022). This diversity originates from the complex interactions of genetics, environmental conditions, and the specific pathogen strains or pests affecting the plant (Liu et al., 2023). Some pea varieties are less susceptible to disease, while others exhibit a high susceptibility (Joshi et al., 2022; Liu et al., 2023).

The resistance of garden peas against fungal diseases has been established by various researchers (Joshi et al., 2022; Dutt et al., 2020). This may be credited to certain genes that hinder the infection (Deb et al., 2020). For instance, a study by Sulima Zhukov (2022) indicated that *P. sativum* exhibited a high resistance against powdery mildew due to the gene MLO1 (Er1). Studies have also evaluated garden pea resistance against many fungal pathogens, such as rhizosphere (Wohor et al., 2022). To create sustainable production of garden peas, studies should be expounded to cover a variety of indigenous *P. sativum* cultivars in Kenya. These varieties include Grano, Kienyeji, Kigondoro, and Kiritho, susceptible to many diseases.

Ascochyta blight disease caused by a group of fungal pathogens (*Ascochyta pinodes*, *Ascochyta pinodella*, *Ascochyta pisi*, and *Phoma koolunga*), is one of the top threats to garden pea (*Pisum sativum*) productivity.

This study intrinsically examines the susceptibility of different pea varieties, such as Grano, Kienyeji, Kigondoro, and Kiritho, to *Ascochyta* blight. These pea varieties include indigenous and traditional varieties, most of which have varying degrees of susceptibility to the disease (Keirnan et al., 2020). Certain pea varieties are naturally resistant, while others are more prone to disease (Sulima & Zhukov, 2022). There is a gap in the research on the level of susceptibility of these *P. sativum* varieties to *Ascochyta* blight. Carrying out such variety resistance profiles evaluation is vital in ceating a sustainable agricultural system as emphasized by Keirnan et al. (2020), is vital, especially in regions with high susceptibility to *Ascochyta* blight, as it may limit their suitability for planting (Joshi et al., 2021).

Knowing the level of the susceptibility of pea varieties to *Ascochyta* blight will impact great practical value for disease prevention (Barbetti et al., 2021). By knowing which varieties of peas are naturally resistant, the breeding plans can be built on this foundation. Resistant varieties can be incorporated into breeding programs to generate novel pea cultivars that overcome *Ascochyta* blight (Lee et al., 2023). New age techniques such as marker-assisted breeding help speed up the production of plants resistant to diseases (Sharma et al. 2023).

Armed with the power of information on variety susceptibility, farmers would implement sustainable disease management systems to foster production (Li et al., 2019). The judicious rotation of varieties less susceptible to disease with more susceptible ones can contribute to a disease burden drop in the fields (Joshi et al., 2022). Moreover, choosing varieties selectively for the local prevalence of diseases and environmental conditions will have a role in optimizing disease management strategies (Khulbe & Sharma, 2020). Integrated disease management (IDM) typically employs resistant varieties alongside fungicides and cultural practices (Riccioni et al. 2019; Anishkumar et al. 2022). Knowing the best varieties to be integrated with these methods is equally important to maximize efficient production. For example, highly susceptible varieties may require more frequent fungicide applications. Equally, more resistant varieties might need less treatment (Barbetti et al., 2021).

Analysis of garden pea fields by detecting *Ascochyta* blight symptoms is critical (Keirnan et al., 2020). The information on variety susceptibility may be used as a driving factor for early detection where efforts are directed at susceptible varieties that may have a high risk. Early detection makes way for timely intervention, decreasing the spread of disease (Jha et al., 2016).

2. Methodology

2.1 Study area

Ascochyta blight diseased *Pisum sativum* plants were collected from molo subcounty and transported to the microbiology laboratory of Egerton University for confirmation of the disease. The study was conducted at Egerton University's Njoro campus, located in Njoro sub-county, and in Molo sub-county, Kenya. The coordinates for Molo sub-county are latitude $0^{\circ}15'0.00''$ S and longitude $35^{\circ}43'59.99''$ E. Egerton University's location in Njoro sub-county is latitude $0^{\circ}22'20.84''$ S and longitude $35^{\circ}56'0.67''$ E.

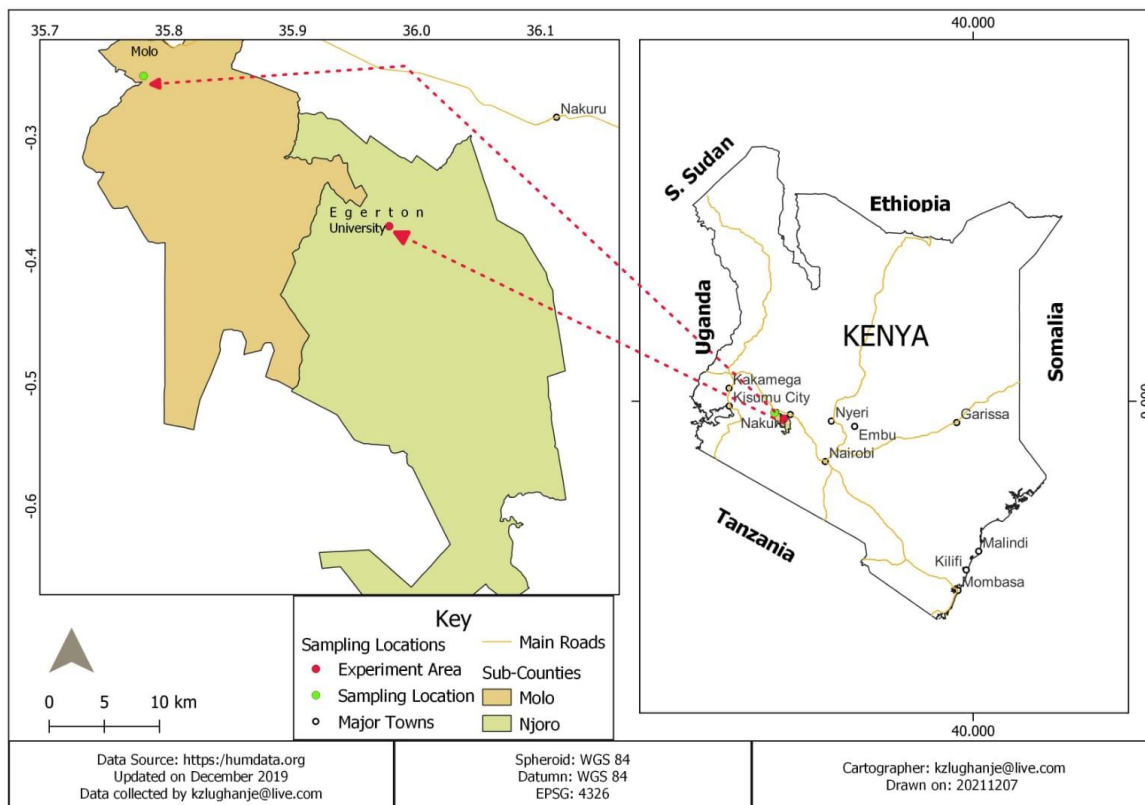


Figure 1: Map showing experimental and sampling locations

2.1.2 Sampling

Diseased *Pisum sativum* plants were selected as the primary source for isolating Ascochyta blight pathogens in the Molo sub-county. Plant samples showing typical symptoms of Ascochyta blight were collected from a farm with a history of the disease within the sub-county. The four garden pea varieties were obtained from the market in Nakuru town for trials in the Molo sub-county. The sampling was carried out between October 2022 and February 2023.

2.1.3 Experimental design

Garden pea varieties were selected for the study, and the experiment was conducted in the field in Molo Sub-county. The study followed a Randomized Complete Block Design (RCBD) with four replicates for each treatment (Table 1).

Table 1: Experimental design

	Treatment 1			Treatment 2	
Rep 1	V1	V2	Rep 1	V1	V2
	V3	V4		V3	V4
	Treatment 1			Treatment 2	
Rep 2	V1	V2	Rep 2	V1	V2
	V3	V4		V3	V4
	Treatment 1			Treatment 2	
Rep 3	V1	V2	Rep 3	V1	V2
	V3	V4		V3	V4

Key

Treatment

Varieties

Treatment 1

Control (Carbendizim) V1

Kienyeji

Treatment 2

Water

V2

Kigondoro

V3

Kiritho

V4

Grano

2.1.4 Isolation and identification of Ascochyta Blight pathogens

Diseased *Pisum sativum* plants were obtained from fields with a history of ascochyta blight disease in Molo sub-county, Nakuru county and brought to the Microbiology laboratory at Egerton University in the Department of Biological Sciences for pathogen isolation and identification. The infected leaves and pods were used to isolate the disease-causing pathogen according to the procedure by Thilagam *et al.* (2018), with some slight adjustments. The samples were surface sterilized by immersing the infected leaves and pods in 70% alcohol and rinsed with running distilled water to remove the alcohol. The leaves and pods were cut into small pieces containing both the diseased and healthy parts. The pieces were then placed on potato dextrose agar (PDA) supplemented with 5g/ml of chloramphenicol to inhibit the growth of bacteria in respective Petri dishes and incubated at 25°C for three weeks.

Ascochyta blight pathogens were identified according to the following characteristics: colony colour on potato dextrose agar (PDA), the morphology of hyphae, presence or absence of chlamydospores, the morphology of sporangium, sporangiophore, antheridia and oogonia.

2.1.5 Purification and cultural conservation

The isolated fungi were conserved using Petri dishes containing potato dextrose agar (PDA) medium. Confirmed single colonies of the isolates were transferred to fresh PDA medium for purification. The purification process was conducted under aseptic laboratory conditions. The purified isolates were maintained on PDA medium at a temperature of 20±2°C. Purified single mycelial colonies were stored on PDA at 4°C, following the protocol described by Ahmed *et al.* (2015).

2.1.6 Screening of garden pea for resistance

Grano, Kigondoro, Kiritho, and Kienyeji garden pea varieties were planted in a field with a history of ascochyta blight disease for examination of ascochyta blight disease symptoms. The disease symptoms were examined after three weeks of growth and included; purple, black, or brown spots, lesions on leaves and pods, blight on lower leaves, and sunken pod lesions, plant withering and desiccation from the base upwards under severe infection as described by Keirnan *et al.* (2020). After the first examination, visual rating of disease was performed every seven days following the DSI (Disease

Severity Index) table, to determine the susceptibility of the different varieties. Plants sprayed with carbendazim were used as negative controls.

After three weeks from the time of inoculation, a visual rating of disease was performed (Table 1) on the experimental plants.

Table 2: Disease Severity Index Table

Disease rating	Description
0	No lesions
1	Minute lesions
2	Lesions up to 5-10mm and slight drooping of apical stems
3	Lesions obvious on all parts of the plant
4	Lesions on all plant parts, defoliation (drying of the leaves) and break the stem

2.7 Data analysis

To determine the susceptibility of *Pisum sativum* varieties to ascochyta blight disease, data was collected and analyzed based on disease severity index table (DSI) observations. Data collected was analysed using analysis of variance (ANOVA) and means separated using the least significant difference (LSD) test at a 0.05 level of significance.

3. Results

3.1 Identification and collection of *Ascochyta* blight-affected *Pisum sativum* plants

Symptoms observed included purple, black, or brown spots and lesions apparent on both leaves and pods. Lower leaf blight and sunken lesions on pods were also observed as typical characteristics of *Ascochyta* blight disease. These symptoms escalated, eventually leading to the withering and desiccation of plants originating from the base and progressing upwards, signifying the disease proliferation process, as displayed in Figure 2.

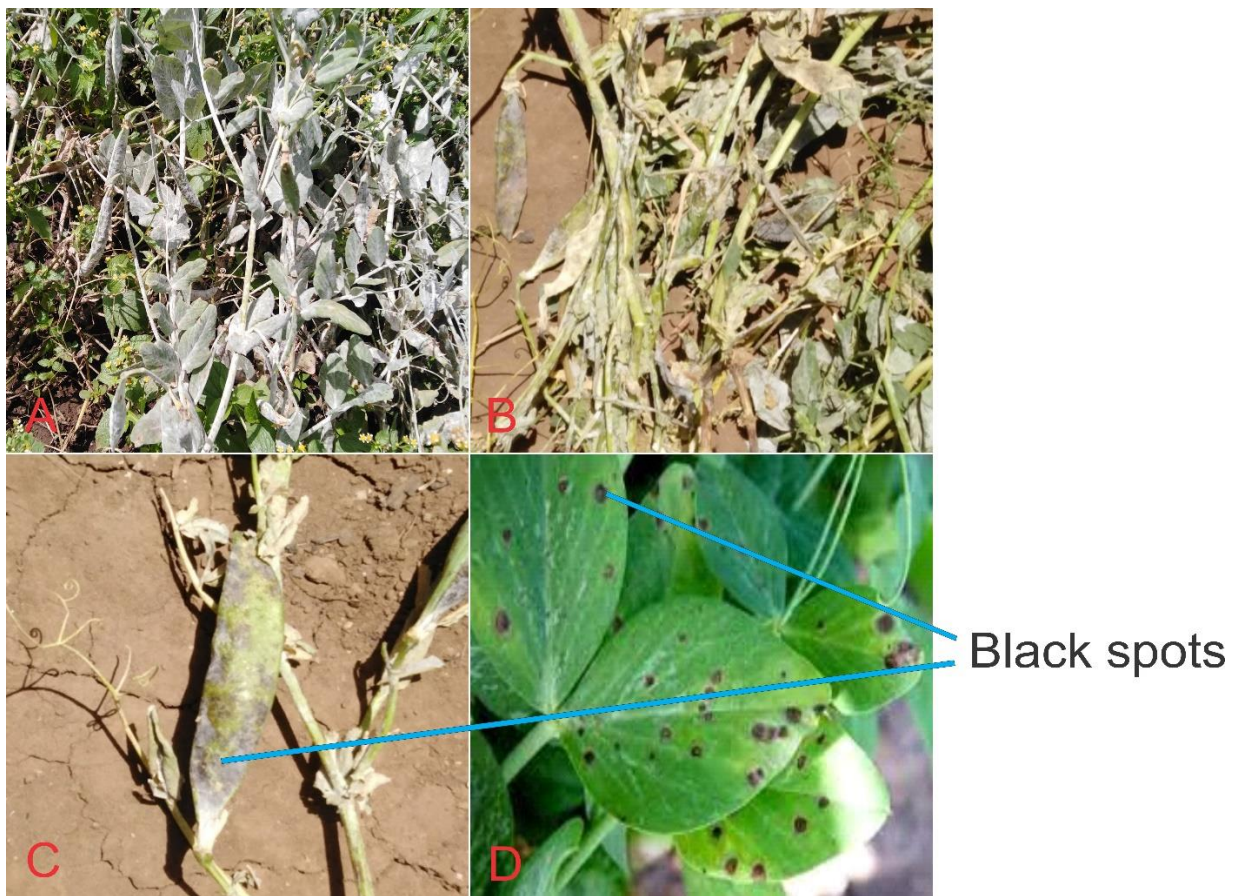


Figure 2: Garden Pea (*Pisum sativum*) Plant, (A) healthy garden pea (*P. sativum*) plant, (B) diseased garden pea (*P. sativum*) plant, (C) pea (*P. sativum*) pod with black spots and (D) symptomatic leaves with black spots

3.2 Identification of *Ascochyta* blight pathogens

On potato dextrose agar (PDA) at 28°C, colonies of *Ascochyta* spp. Exhibited a colour spectrum ranging from white to grey and a woolly texture characterized by irregularly defined edges after seven days of

growth. The reverse view showed a concentric growth pattern (Plate 1). Microscopic examination revealed septate hyphae and spherical sporangia.

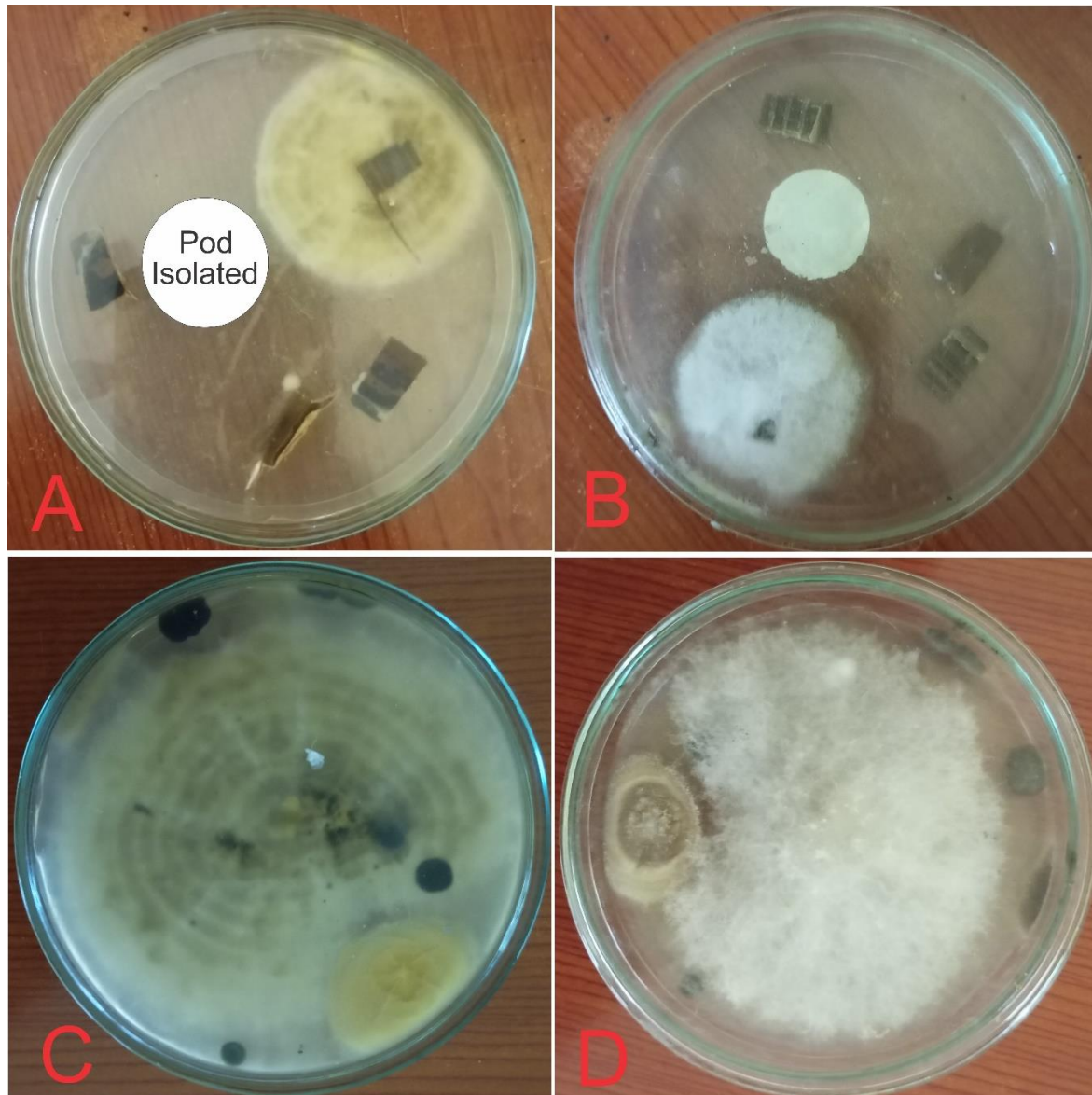


Plate 1: *Ascochyta* spp. colony growth on PDA (A) reverse view after 8 days, (B) direct view after 8 days, (C) reverse view after 14 days, (D) direct view after 14 days

3.3 Susceptibility of pea varieties

The Kienyeji and Kigondoro varieties exhibited the highest susceptibility to *Ascochyta* blight disease, with mean severity scores of 2.83 ($S = 0.857$) and 1.098 ($S = 0.857$), respectively. Following closely in susceptibility was the Kiritho variety, with a mean severity score of 2.78 ($S = 1.166$). Conversely, the Grano variety demonstrated the lowest susceptibility, recording a mean severity score of 2.06 ($S = 1.056$). These findings offer valuable insights into the relative susceptibility of each variety to

Ascochyta blight disease, with Kienyeji and Kigondoro being the most susceptible, followed by Kiritho, and Grano displaying the least susceptibility among the experimented varieties (Figure 3).

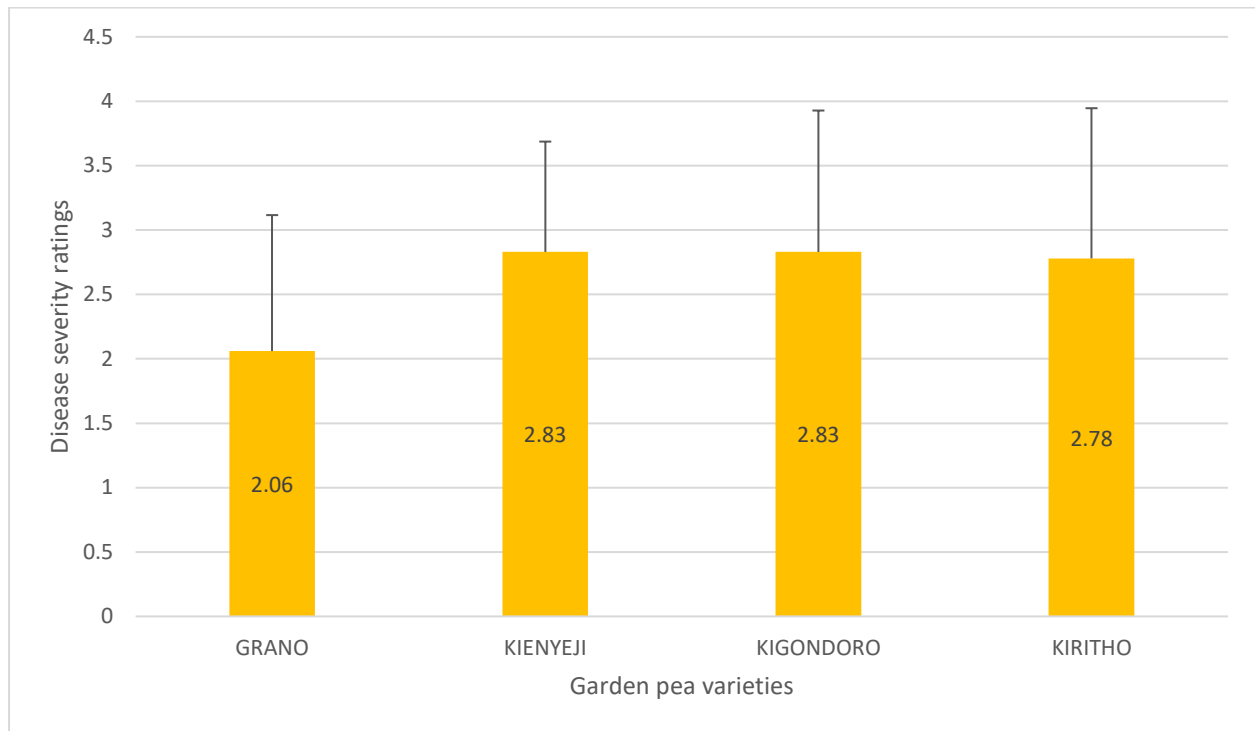


Figure 3: Bar plot showing disease severity means and standard deviation of four garden pea varieties affected by ascochyta blight

Upon conducting the one-way ANOVA analysis, a p-value of 0.079 was obtained. Failing to reject the null hypothesis due to p-value exceeding the chosen significance level of 0.05 indicates insufficient evidence to conclude a significant difference in resistance to Ascochyta blight pathogens among the four pea varieties (Grano, Kienyeji, Kigondoro, and Kiritho) regarding Ascochyta blight disease susceptibility.

However, Fischer's Least Significant Difference (LSD) test revealed significant differences in the susceptibility means of the Grano pea variety compared to the Kienyeji ($p = 0.030$), Kigondoro ($p = 0.030$), and Kiritho ($p = 0.043$) pea varieties. There was no significant difference between the disease severity score means of the three varieties; Kienyeji, Kigondoro, and Kiritho, as all surpassed the set confidence level of 0.05. These findings signify that the Grano pea variety exhibited lower susceptibility to Ascochyta blight pathogens than the Kienyeji, Kigondoro, and Kiritho pea varieties.

Conversely, no significant difference in disease severity scores were evident between the latter three varieties

4.0 Discussion

4.1 Signs and symptoms of ascochyta blight disease in Garden pea

Some of the symptoms related to Ascochyta blight disease from the study included purple, black, or brown discoloration of the leaves and pod tissues alongside the formation of lesions on the leaves and pods. Blight on the plant's lower leaves and sunken lesions of pods were also recorded and are in tandem with studies conducted by Hafiz and Ahmed (2022). These symptoms developed into withering and desiccation of plants, starting from the base and moving up along the stems, thus showing the progression of the disease throughout the parts of the plant. The observed symptoms and pathogenic properties of Ascochyta blight are consistent with previous studies (Liu et al., 2016; Keirnan et al., 2020; Gossen et al., 2023). The progression of the disease from leaf spot to a severe lesion on pods and subsequent plant desiccation sheds more light on the pathogenic potential of ascochyta blight pathogens affecting all plant varieties, including chickpeas (Mart et al., 2022). Such a disease pattern suggests a systemic infection that weakens plant vigor from the base, as observed with known disease development in the affected crops (Keirnan et al., 2020).

Consequently, these findings confirm that Ascochyta blight imposes a formidable threat to crop yield and quality. Knowledge of these disease symptoms is critical in early detection, which will aid the application of intervention measures (Gudero Mengesha et al., 2022). Identifying these characteristic symptoms can be helpful in future research endeavors geared toward identifying new management practices that might lessen crop losses (Richard et al., 2021).

However, although one can identify critical symptoms, the actual degree of yield loss alongside other factors that determine the severity of the disease in the environment should be comprehensively investigated (Ngoune Liliane & Shelton Charles, 2020; Ristaino et al., 2021). Further studies must explore the host plant resistance, especially gene resistance and molecular characterization of fungal pathogens that cause the disease (Kaur et al., 2022). Consequently, further research should be directed toward identifying the losses incurred due to Ascochyta blight, genetic resistance in various plant genotypes, and molecular characterization of the pathogen.

4.2 Susceptibility of pea varieties

This study focused on determining the extent to which different types of pea varieties are vulnerable to *Ascochyta* blight, which is a significant menace to the production of garden peas (*Pisum sativum*). Among all the varieties, the Kienyeji and Kigondoro varieties were identified as the most susceptible, having mean disease severity scores of 2.83 ($S = 0.857$) and 1.098 ($S = 0.857$), respectively. The mean severity score for Kiritho was 2.78 ($S = 1.166$), while Grano was at 2.06 ($S = 1.056$), indicating that it was the least susceptible to the disease. The outcomes demonstrated a high variability in susceptibility to *Ascochyta* blight.

As such, the implications arising from these findings are profound and broad-spectrum and correspond with the research by Boros & Wawer (2009) and Olle & Sooväli (2020). Notably, the findings provide information about the reaction of various pea cultivars to *Ascochyta* blight disease. With this knowledge, farmers can make the right growing choices, considering each variety's disease resistance levels (Pankou et al., 2022). For instance, screening for less susceptible varieties, such as Grano, with the least susceptibility to the disease, is vital in reducing yield losses, which aligns with the literature in supporting disease-resistant varieties as crucial to sustainable crop production (Barbetti et al., 2021; Bigini et al., 2021; Wohor et al., 2022).

These findings hold many implications, especially for farmers, agronomists, and plant breeders, given that the information from this study offers a method that would enable the selection of pea varieties based on their level of susceptibility to *Ascochyta* blight, whereby such varieties can be used in the regions where the disease is most dominant (Bigini et al., 2021). For example, growers in the Molo sub-county where *Ascochyta* blight is endemic could enjoy planting the Grano variety tagged as the least susceptible. Such a choice helps avoid severe yield losses due to this disease and increases both the yields and the economic returns. Moreover, these results contribute to the broader understanding of Integrated Disease Management (IDM) strategies for *P. sativum* (Kumar et al., 2020). However, the high resistance of the Grano variety does not preclude the cultivation of the other three varieties despite their relatively higher susceptibility. Instead, it suggests that these varieties could still be successfully grown in *Ascochyta* blight-prone areas with proper management practices. Implementing IDM practices such as crop rotation, fungicide application, and adjusting sowing dates could help mitigate the impact of the disease on these more susceptible varieties (Ul Haq et al., 2020; Degani et al., 2022).

However, some management methods require more assessment; for example, the belief that liming could reduce *Ascochyta* blight and increase other diseases' vulnerability, such as Downy mildew (Rhouma et al., 2023), shows the need for a multifaceted approach that includes a variety of diseases

and how they interact (Barbetti et al., 2021). Where IDM strategies are primarily implemented, recognizing the variation of one type of pea to the other to ascochyta blight disease ensures that each attribute is considered, therefore enhancing the level of disease management.

One of the significant sources of limitations of this study was solely dependence on disease severity scores while negating crop yield data. Knowledge of Ascochyta blight severity and how it affects yields is critical to estimating the disease's economic importance (Durham & Mizik, 2021). Thus, there is a need to perform more research to establish a broader application of disease resistance, which was noted in the study. For instance, a variety that possesses moderate to disease resistance but high yield capacity can be economically more viable than one with a highly disease-resistant capacity but less yield. Also, there is a need for the research to extend to other legume agronomic factors, including soil nutrient status, climate change, or other existing pests and diseases that can affect disease yield and crop performance (Richard et al., 2021; Rempelos et al., 2021). All these factors are essential for comprehending the composite picture of disease resistance and formulating suitable management measures.

5.0 Conclusion

In summation, the findings of this study illuminate the divergent Susceptibility of distinct pea varieties to Ascochyta blight disease. These findings contribute to the existing reservoir of knowledge concerning the diversity within pea varieties and their varied resistance to diseases. The results carry practical implications for farmers, guiding them in selecting varieties with heightened disease resistance and ultimately bolstering the overall sustainability of pea cultivation. Furthermore, the findings underscore the paramount importance of IDM strategies that thoughtfully integrate the unique characteristics of each variety. By accentuating the significance of disease resistance in the selection and management of crops, this study aligns seamlessly with the broader objective of advancing agricultural sustainability and productivity.

Ultimately, the study shows that Ascochyta blight is a genuine concern for garden pea production since different varieties exhibited different levels of susceptibility. The findings underscore that although varieties like Grano have a higher comparative resistance to Ascochyta blight, other varieties such as Kiritho, Kigondoro, and Kiiyenyeji may be grown extensively in disease-affected areas if IDM tools are considered. There is a need for research on Ascochyta blight effects on the economy, the variety and genetic control of the resistance in various genotypes of the plant, and the interaction within the biological factors, including the impact of the soil, climate change, and pests, for the formulation of effective and sound management procedures. This present investigation forms a

background for choosing disease-resistant peas to promote improved yields and minimized yield losses in regions influenced by *Ascochyta* blight.

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