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Symptomatology and Identification of Major Fungal Pathogens of Pyrethrum (*Chrysanthemum cinareriifolium)* in Kenya

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Abstract

Fungal pathogens are major biotic constraints in almost all pyrethrum producing zones in the world. Due to limited information, this study was designed to document on symptomatology and diversity of major fungal pathogens of pyrethrum in Kenya. Stratified random sampling technique was applied during a field sampling in major production zones of Nakuru County where agro ecological zones were considered as strata and on each stratum, biased sampling was used to sample pyrethrum tissues showing symptoms of fungal infections. Disease symptoms on these samples were described based on the findings of earlier scholars. The samples were taken to University of Eldoret Plant Pathology Laboratory for fungal isolation, morphological studies and identification. Surface sterilized samples were cultured on PDA media and incubated at 25 ºC - 27 ºC for seven days after which the actively growing fungal mycelia were sub cultured to obtain pure isolates on a freshly prepared PDA and incubated at alternating 12 hours of light and darkness to induce sporulation. A light microscope was used at X1000 magnification to assess micro characteristics while Color charts were used to describe macro characteristics of isolates and laboratory manuals, journals and reference books were used to confirm the actual identities of each isolate. Frequency of occurrence was assessed based on sites as well. Three major diseases were described in both sites; Bud disease, crown rot disease and pyrethrum wilt disease. A total of ten diverse pure isolates belonging to six different genera were obtained from the samples analyzed and from these isolates, genus *Fusarium* was the most abundant with four different species namely *F. oxysporum*, *F. solani, F. graminearium* and *F. avenaceum*. Followed closely by the genus *Alternaria* with three species isolated and they include *Alternaria solani*, *Alternaria alternata* and *Alternaria tenuissima.* Other identified fungi were *Rhizoctonia solani*, *Phoma* spp and *Sclerotinia minor*. It is evident that a wide range of fungal pathogens are responsible for low pyrethrum yield and pyrethrum quality in Kenya thus the need to exploit effective management strategies.

Keywords: *Symptomatology, identification, fungi, pyrethrum*

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1.0 Introduction

Pyrethrum (*Chrysanthemum cinareriifolium*) is a perennial daisy-like plant which belongs to the *Asteraceae* family and is one of the major cash crops grown in Kenya which is mainly cultivated purposely for production of pyrethrin, an organic insecticide that acts against a wide range of insect pests (Bhuiyan *et al*., 2019; Sun *et al*., 2020). There has been a sharp decline in pyrethrum production in Kenya and this has been attributed to biotic,

abiotic as well as the socio economic factors. In the past four decades, Kenya was a leading producer of pyrethrum and was supplying about 70% of the worlds pyrethrin in the global market but by 2018, the supply had dropped to about 2% (Matonda, 2018) and fungal pathogens are among the major contributors to decline in production in Kenya and other parts of the world (Shimira *et al*., 2021).

Since the introduction of pyrethrum production, several plant pathogens have been reported to cause diseases and emergence of new races has occurred over the years in different agro ecological zones of the world, For instance, true and false bud disease was first reported in Kenya in the year 1946 (Shimira *et al*., 2021), Anthracnose disease was reported in Australia in the year 2013 (Barimani *et al*., 2013), ray blight was reported in the year 1995, *Rhizoctonia solani* was identified to cause root rots between the year 1999-2000 (Alam *et al*., 2006). However, very limited research especially on microscopy and identification of possible pathotypes evolving from evolutionary mechanisms has been done.

In terms of quantified yield loss, fewer diseases have been documented to cause significant damage to pyrethrum. For instance, pyrethrum wilting caused by *Fusarium* spp. can cause up to 50% yield losses (Pereira *et al*., 2019; Singh, 2014) while *Sclerotinia spp. and Rhizoctonia spp.* have been reported to cause wilting of pyrethrum (Cubeta & Vilgalys, 1997; O'Malley *et al*., 2015). In addition, anthracnose disease can cause up to 67% loses on susceptible varieties (Huang *et al*., 2022), while ray blight which is the major disease in major pyrethrum growing zones can cause up to 100 % yield losses in production of flowers (Bhuiyan *et al.*, 2019).

The evolutionary nature of a number of fungal pathogens indicates that environmental conditions greatly influence their distribution and that there is a possibility of emergence of new races of pathogens over the years with majority of pathotypes emerging as a result of introduction of new host plant with a new mechanism known as host-pathogen differentiation mechanism. However, despite the availability of different varieties of pyrethrum coupled with varying environmental conditions in Kenya, there is no current documentation on the status of fungal pathogens affecting pyrethrum production in Kenya hence the need for regular monitoring and identification of pathotypes to widen knowledge in development of not only effective but also sustainable management strategies.

2. Materials and methods

2.1 Site of experiment and site characteristics

A field survey and tissue sampling in major pyrethrum growing zones in Nakuru County was done where stratified random sampling technique was used and two sites Subukia and Molo were considered as strata. These sites lies on different agro ecological zones representing different regions in Kenya. Molo is situated along the Mau forest within the Mau escarpment at coordinates: 0° 15' S and 35° 44' E. This site is a high altitude zone of between 2,980 to 3,050 meters above sea level whereas annual rainfall ranges between 1,200 to 1,900 millimeters per year, while annual temperature ranges between 8 °C to 23 °C in this site. Subukia on the other hand lies at a latitude 0° 0' 6.7705" S and longitude 36° 13' 40.1682" E and is located at a low altitude zone of between 2,010 to 2,295 meters above sea level and is characterized by an average annual temperatures of between 10 °C to 26 °C and rainfall of between 700 to 1,400 millimeters per annum (Kinyori, 2016; Muturi *et al*., 2018).

2.2 Infected tissue sampling

From every stratum (site), biased random sampling was used to collect infected pyrethrum tissues showing symptoms of fungal infection which were examined and their symptoms recorded. These samples were carefully

detached from the mother plants and placed on small 'khaki' bags, labeled and transported to University of Eldoret plant pathology laboratory where they were stored at 4 ºC before isolation of the fungi. Among the sampled plant parts were roots, crown, stems, leaves and flowers.

2.3 Isolation of fungal pathogens and culture purification

Two hundred grams (200 g) of clean potatoes were pilled, sliced into smaller pieces and boiled in 500 ml sterile distilled water for 20 minutes. The resultant infusion was filtered using a muslin cloth to remove all potato flesh that might have accompanied the filtrate, and then 20 grams of dextrose and 15 grams of agar were added to the filtrate simultaneously and heated while stirring using a magnetic stirrer to ensure that dextrose and agar dissolved uniformly. The resultant mixture was topped up to 1 liter and sterilized in an autoclave for 15 minutes at 121 ºC temperature and approximately 15 PSI (pounds per square inch) pressure. The hot media was then dispensed onto medium sized glass petri dishes a laminar airflow chamber and allowed to cool before use (Kipkogei, 2019).

Approximately 2 to 5 mm^2 of infected pyrethrum tissue from the edge of an actively growing lesion of fresh samples were excised and sterilized using 1% sodium hypochlorite solution for 30 seconds and rinsed three times with sterile distilled water to remove traces of sterilizing agent. Sterilized samples were then plated onto plates containing PDA in a laminar flow cabinet and incubated at 24 ºC to 28 ºC for seven days. From each petri-dish, small portions of all different fungal colonies that grew were purified by transferring the mycelial portions to freshly prepared PDA and incubated under an alternating 12 h near-UV light/12 h dark photoperiod for 14 days to induce sporulation. Pure fungal colonies were then studied, described and identified through microscopy at X1000 magnification with the help of pathology journals and reference books (Kipngeno, 2015).

2.4 Assessment of diversity and frequency of isolation

Pure isolates were identified to species level using morphological and microscopic characteristics. Specifically, substrate pigmentation and mycelial color as well as mycelial growth characteristics were described according to pathology journals and reference books while conidial shapes and sizes were determined using a microscope (Yassin *et al*., 2011).

Frequency of isolation was assessed as the number of times every isolate has appeared per site sampled. As described by; (Benard *et al*., 2013) using the following equation;

$$\textbf{Frequency}\ (\%) = \frac{Number\ of\ isolates\ of\ a\ genus}{Total\ number\ of\ all\ isolates}\ \text{x}\ 100$$

3.0 Results

Different plant parts such as crown, flowers, leaves and stems were found to show symptoms of fungal disease infection which were common across the zones and were identified as bud disease, pyrethrum wilt and crown rot diseases. A total of 10 isolates were identified from the zones sampled and distinct morphological differences were observed physically on plates and at microscopic level in terms of spore shape and size as well as mycelial characteristics. *Fusarium oxysporum, Fusarium solani, Fusarium graminearium* and *Fusarium avenaceum* were found to be the major pathogens causing pyrethrum wilt disease. Also, *Sclerotinia minor*, *Alternaria solani* and *Rhizoctonia solani* were isolated from samples with crown rot symptoms while *Phoma* spp., *Alternaria alternata* and *Alternaria tenuissima* were found in tissues showing symptoms of bud disease and there is need to confirm which specific pathogen was responsible for the observed symptoms.

3.1 Pyrethrum wilt

Pyrethrum wilt disease was diagnosed as described by previous researchers (Kimani *et al*., 2001; Kinyua, 1996; Nattrass, 1950; Njoya-Kimani, 2001) based on the symptoms such as chlorotic leaves on some plants, beginning with the lower leaves and progressing to the leaf petioles as the mid veins gradually turn yellow, Upon successful infection, affected pyrethrum display drooping of leaves as the disease progresses then wilting and eventually death of the whole plant as shown in Plate 1 (a). Brown discoloration on stems as a sign of developing vascular colonization was observed as shown in Plate 1(b) as well as pale bleached lesions on stems that turn yellow and eventually dry as shown in Plate 1 (c).

(a) **(b)** **(c)**

Plate 1(i): Pyrethrum plant parts showing symptoms of wilt disease: (a) yellowing of leaves and Leaf petioles, (b) brown discoloration on stems, (c) yellowing and drying of stems

From the wilted parts as shown in Plate 1(a), 1(b) and 1(c) above, four fungal species were identified belonging genus *Fusarium*: *Fusarium oxysporum*, *Fusarium solani*, *Fusarium avenaceum* and *Fusarium graminearium* as shown in Plate 1(ii) below. These pathogens were isolated from different plant parts that included leaves, stems, flower stalk and even flower heads that were showing symptoms of wilt disease. Macroscopic as well as morphological characteristics were used in identifying these pathogens (Arie, 2019; Machado *et al.*, 2015; Moslemi *et al.*, 2017b; O'Malley, 2012).

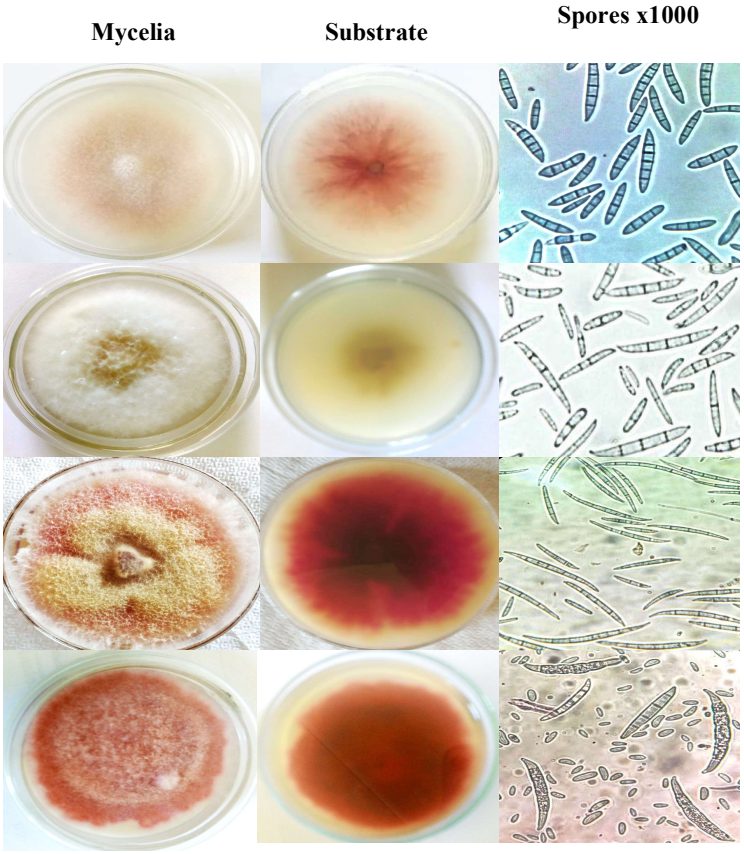

Plate 1(ii): *Fusarium* species isolated from samples with pyrethrum wilt disease *(Fusarium oxysporum), (Fusarium solani), (Fusarium avenaceum)* and *(Fusarium graminearium).*

3.2 Crown rot disease

This disease is characterized by dark, brown necrotic lesions on roots and the other basal parts of the plant such as the crown. These lesions enlarge as the disease progresses to the advanced stages resulting to wilting of the entire plant and in some cases; the crown part of the plant starts rotting and lower leaf petioles starts detaching themselves from the mother plant. (Cubeta & Vilgalys, 1997) as shown in Plate 2(i) below:

Plate 2(i): Upper parts of pyrethrum roots affected by the crown rot disease

For the crown rot diseased samples, three major fungal pathogens were identified: *Rhizoctonia solani, Alternaria solani* and *Sclerotinia minor* Plate 2(ii) below. These species belongs to three different genera and were identified based on their macro and micro characteristics as described by (Al-Fadhal *et al*., 2019; Alhussaen, 2012; Moni *et al*., 2016; Moslemi *et al*., 2016) . Other species identified with lower frequency of occurrences were: *Fusarium oxysporum* and *Fusarium solani*.

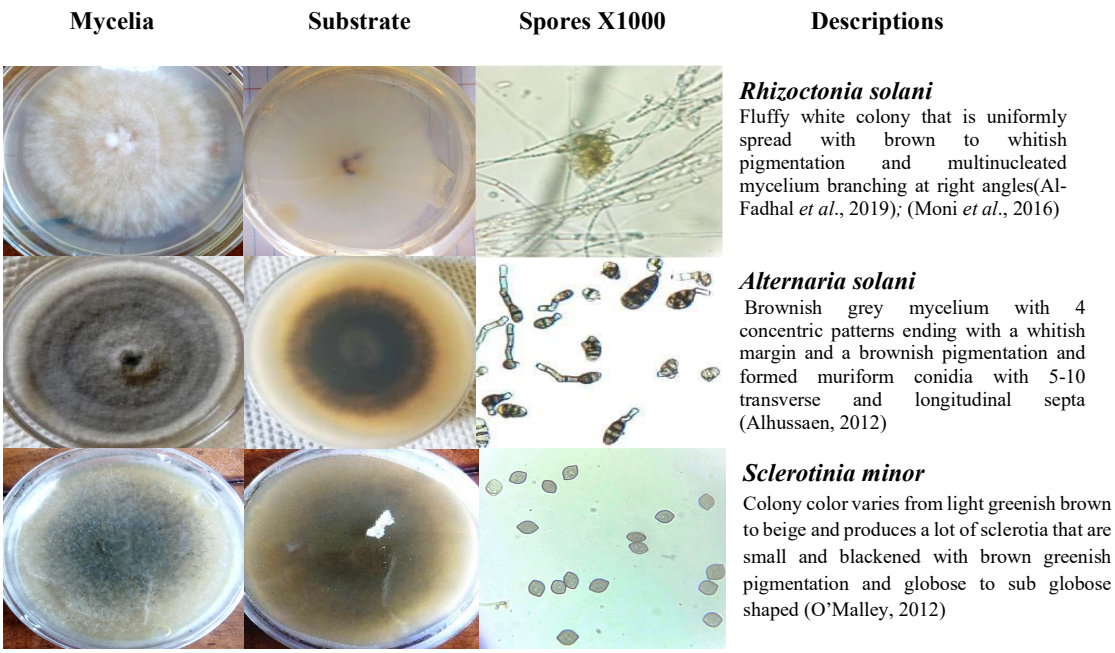

Plate 2 (ii); Fungal species isolated from pyrethrum plant infected with crown rot disease

3.3 Bud disease

This disease was identified following the ray florets' blighting symptoms, which cause the heads to turn straw-colored or wilted Plate 3(i)). The most recognizable sign of bud disease was the emergence of flower buds with a "shepherd's crook," symptom which is brought about by infection and necrosis on one side of the higher flower stem (2 to 3 cm below the flower bud) (Bhuiyan *et al*., 2019).

Plate 3(i): Pyrethrum flowers with symptoms of Bud disease

Infected tissues showing symptoms of bud disease produced three pathogenic fungi namely *Alternaria alternata, Alternaria tenuissima* and *Phoma* spp following the macro characteristics and micro characteristics. (Hay *et al*., 2015; Meena *et al*., 2017; Moslemi *et al*., 2017a; Vaghefi *et al*., 2012) as shown in Plate 3(ii) below.

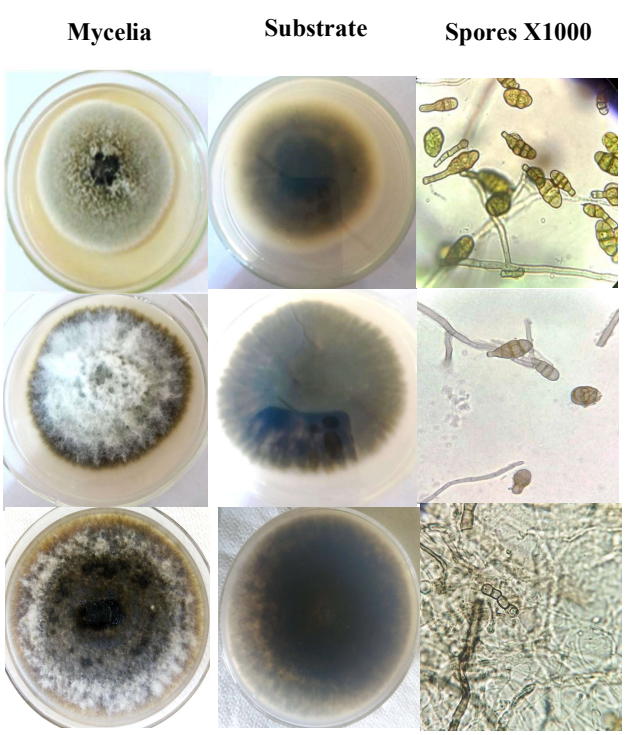

Plate 3 (ii): Isolates associated with bud disease of pyrethrum; *Alternaria alternata, Alternaria tenuissima* and *Phoma* spp.

3.2 Frequency of isolation of identified fungal pathogens

The genus *Fusarium* was the most diverse genus with four species isolated; *F. oxysporum*, *F. Solani*, *F. avenaceum* and *F. graminearium* followed by genus *Alternaria* with three species isolated; *A. solani*, *A. alternata* and *A. tenuissima*, while *Phoma* spp., *Rhizoctonia solani* and *Sclerotinia minor* were single species isolated from specific genera. Seven species were isolated from Subukia while ten species were isolated from Molo site with *F. graminearium, S. minor* and *A. solani* confined to Molo site only. A total of sixty isolates were obtained from Subukia site samples while eighty species were isolated from Molo site samples. With respect to specific pathogens, *F. oxysporum* recorded a highest isolation frequency followed by *F. solani* while *Phoma* spp. and *F. graminearium* had the lowest isolation frequencies (Figure 1).

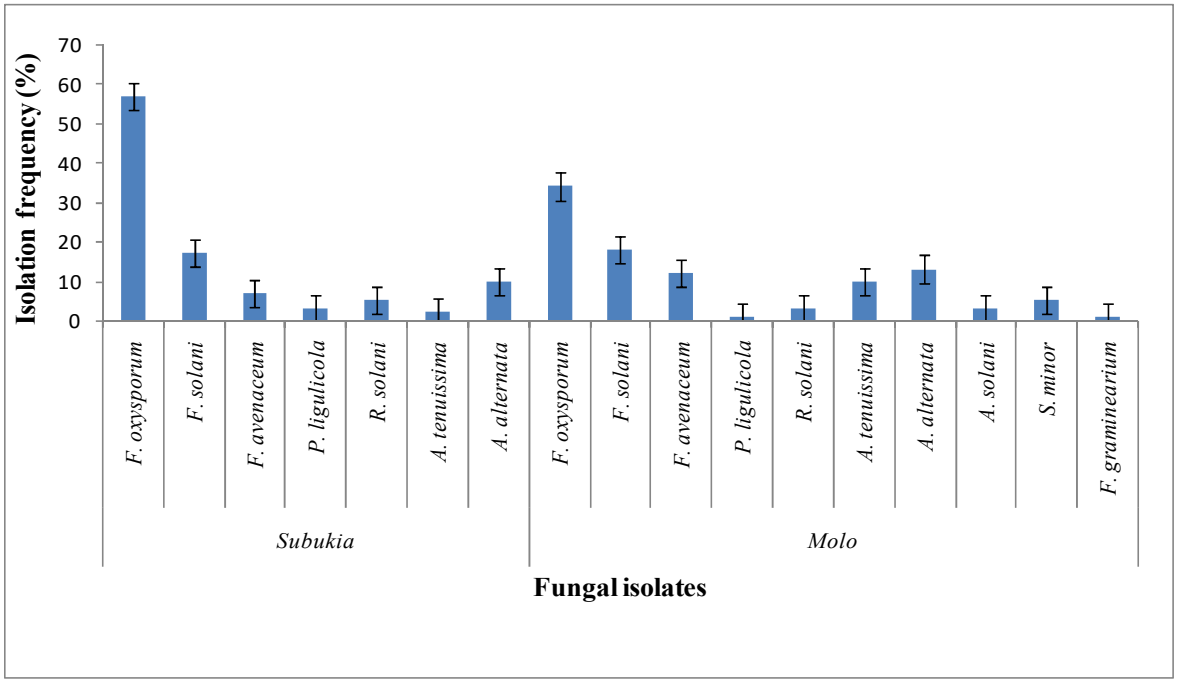

Figure 1: Isolation frequencies of fungal species isolated from Subukia. Error bars represent standard errors

4.0 Discussion

Pyrethrum wilts, crown rot and bud disease were the major fungal diseases identified in the fields sampled. In susceptible varieties, wilt causing pathogens enters the plant tissues passively or actively through direct penetration by development of hyphae under favorable conditions. This hyphae enters the epidermal cells and grows infecting the vascular tissues and blocks the xylem vessels hindering water flow resulting to wilting of the plant (Moslemi, 2017). Crown rot fungus enters the plant through roots and others directly entering the crown. These fungi secretes some enzymes that degrades the cell walls resulting to soft plant tissues that are vulnerable to rotting, darkening and eventually dying when exposed to harsh environmental conditions (Kader, 2002). Bud disease was characterized by emergence of flower buds with a "shepherd's crook," symptom as a result of infection of one side of higher flower stem that is two to three centimeters below the flower head. This condition resulted to permanent dead of the flower heads since fungal pathogens blocks vascular tissues (xylem and phloem) thus hindering photo assimilates, water and other nutrients from reaching the flower head (O'Malley, 2012; Pethybridge *et al.*, 2008).

There was a diversity in morphological characteristics of isolated fungal species due to their differences in ecological roles and nutritional modes as well as their modes of reproduction, survival and dispersal (Bahram & Netherway, 2022). Different species of the same genus were isolated from different plant parts sampled and were found to cause different diseases for example *Alternaria* spp or similar disease for the case of *Fusarium* spp; these results could be attributed to their different genetic compositions responsible for their widely immune system responses and thus capable of causing a wide range of diseases or maybe found affecting the same host (Bahram

& Netherway, 2022). *Sclerotinia minor* and *Fusarium graminearium* were confined to Molo site only this could be because of their ability to adapt to environmental conditions of this site.

Differences in isolation frequencies were recorded among the different species and this indicates that there exists variability in terms of virulence of these fungi, favorable environmental conditions and also susceptibility of the host (Singh Saharan *et al*., 2023). *Fusarium oxysporum* had the highest frequency of isolation while *Phoma ligulicola* had the lowest isolation frequency. This means that *Fusarium oxysporum* was the most virulent pathogen and well adapted to prevailing environmental conditions. Farmers' lack of adequate knowledge in the current status of disease pressure, identity and mitigation measures as well as use of susceptible varieties (Liu *et al*., 2021; Moslemi *et al*., 2016; Motanya, 2019; Nyoro, 2019), may have been the main reasons for these results, therefore, this calls for proper management strategies to ensure maximum production of pyrethrum in Kenya.

5.0 Conclusion and recommendations

A total of ten diverse fungal species were identified to cause major diseases including pyrethrum wilt, bud disease and crown rot in major pyrethrum growing zones of Kenya. This study recommends further studies on the response of different pyrethrum genotypes to specific fungal pathogens under virulence and pathogenicity studies. Also, for effective and sustainable management, of the identified fungal pathogens, this work recommends further studies to identify the most efficacious biocontrols and botanical extracts for proper management of each of the fungal pathogen.

6.0 Acknowledgement

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