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Distribution and Status of Citrus Leaf and Fruit Spot (*Pseudocercospora angolensis*) Disease in North Western Ethiopia

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Abstract

Citrus leaf and fruit spot incited by *Pseudocercospora angolensis* are one of the most serious production constraints of citrus production in North Western Ethiopia. Disease survey in 10 major citrus growing districts of North-Western Ethiopia was conducted, to indicate the importance of citrus leaf and fruit spot disease and its association with agro-ecological factors. The study was carried out during 2021 and 2022 cropping seasons. And in each cropping season based on availability, a total of 280 citrus trees (200 sweet oranges, 40 mandarins, and 40 lemons) in 20 orchards were randomly assessed. This study result showed that in most of the surveyed areas, leaf and fruit spot were the most prevalent and long-lasting disease of citrus. That is, among 10 studied districts in 8 of them (100%) disease prevalence, (87.25%) incidence and (24.43%) severity were computed. As a result, most citrus growers were practiced uprooting of their citrus trees and replacing them by other fruit crops (Mango and Avocado). Therefore, from the result of the present investigation to provide sustainable citrus fruit productivity and production in areas where citrus leaf and fruit spot disease is predominant and causes a devastating effect, integrated and sustainable citrus leaf and fruit spot disease management practices should be executed. Furthermore, in order to get full illustrates of the importance of the disease, it is useful to conduct related disease surveys in major citrus growing areas of the country.

Keywords

Identification, Isolation, Infection, Conidia, Survey

1. Introduction

The distribution and infection processes of *Pseudocercospora* fungus are similar to other citrus fungal pathogens whose asexual spores (conidia) are primary sources for new infection. Infection is apparently favored by wind-blown rains that occur when susceptible young leaves and fruit are present and temperatures are warm. Leaf and fruit spot disease of citrus is a great concern for citrus producing areas in warm humid regions like Florida [1] [2]. Its presence in Yemen and the possible spread to Asia is also another potential threat [3].

The development and spread of *Pseudocercospora angolensis*is are favored in areas with high rain fall and humidity above 75%. Besides, under natural conditions, spores of *Pseudocercospora angolensis* are probably spread by air-borne conidia. And leaves of planting material or fruits seem the most likely path-ways for the transfer of the pathogen. Citrus fruits can be attacked at all growing stages, whereas leaves are less affected as they get older [4].

Long distance dispersal of the fungus is by windborne conidia. While spread in the orchards is primarily by means of rain drops laden with spores and rain splash. Humans may be responsible for the inadvertent movement of infected planting material and fruit between areas [5].

Although the survival mechanisms of *Pseudocercospora angolensis* are not well known [6], the fungus is able to survive, probably in dormant lesions on infected material until the onset of conditions conducive to sporulation [2] [7] [8] [9].

Currently, citrus plantations in the south, south-west and north-west of Ethiopia are seriously affected by this disease. Consequently, the supply of citrus fruits in these areas has been insufficient for the last ten years. Several small-scale citrus growers have abandoned their trees due to severe infection of citrus by the disease and also the disease is becoming the major limiting factor of the citrus industry [9]. As a result, diagnosis and regular monitoring of the disease are required for devising sustainable disease management practices thereby reducing the yield loss and improving the quality of citrus fruits [10]. Therefore, this study was anticipated to assess the distribution and status of citrus leaf and fruit spot disease in North-Western Ethiopia.

2. Materials and Methods

2.1. Survey Area and Duration

The study was carried out in 2021 and 2022 cropping seasons in 10 major citrus growing districts of North-Western Ethiopia. And the locations of the surveyed areas fall between longitudes of 37˚35'37"E and latitudes of 11˚27'21"N.

2.2. Sampling and Sampling Units

To select the assessed 20 orchards, a purposive sampling method was applied. And in each cropping season based on availability, a total of 280 citrus trees (200 sweet oranges, 40 mandarins, and 40 lemons) were randomly assessed.

2.3. Disease Assessment

Disease prevalence was determined by the number of orchards surveyed showing the disease symptom, expressed as the percentage proportion of the total number of orchards surveyed [11].

And disease incidence was estimated both on leaves and fruits of the plant. On leaves, it was estimated by counting the visibly infected and total number of leaves on eight randomly selected terminal shoots from the upper and lower halves of the canopy in four directions (North, South, East, and West) of each selected tree, and expressed as a percentage [12]. It was computed by [13] formula as:

$$\text{Disease incidence on leaves}(\%) = \frac{\text{No of leaves infected per tree}}{\text{Total no of assessed leaves per tree}} \times 100$$

On fruits, it was assessed on 5 to 40 randomly selected intact fruits in four directions of each tree based on the presence or absence of visible disease symptoms on each fruit, depending on availability [12]. And it was calculated by using the [13] formula:

$$\text{Disease incidence on fruits } (\%) = \frac{\text{No of infected fruits per tree}}{\text{Total no of assessed fruits per tree}} \times 100$$

Disease severity was assessed on the same leaf and fruit samples that were chosen for disease incidence scoring. On leaves, it was estimated based on a zero-to-four scoring scale, where 0 = no symptoms, 1 = 1% to 25%, 2 = 26% to 50%, 3 = 51% to 75% and 4 = above 75% of leaf area infected [14] [15].

And on fruits, it was recorded using the following zero to four scoring scale, where 0 = healthy, 1 = less than 5%, 2 = 5% to 20%, 3 = 21% - 50% and 4 = above 50% of fruit surface affected [16].

For analysis purposes, severity grades were converted into percentage severity index (PSI) and it was calculated using the following formula suggested by [17]:

$$\text{PSI} = \frac{\text{Sum of all numerical ratings}}{\text{Total no. of observations} \times \text{Maximum disease score}} \times 100$$

To obtain additional information about the occurrence and status of citrus production and management practices of the sampled areas, a questionnaire was used. The questionnaire mainly compiled: The number of citrus trees growing, their species and cultivar types, orchard and/or tree age, soil type, input application, pest infestation history, pest management practices and ownership. All information collected were summarized and described to give an overview of citrus production in North Western Ethiopia. A face-to-face (Personal) interview method was implemented. And depending on the type of ownership the interviewee were Farmers, Investors, and Agronomy experts.

2.4. Sample Collection

In each orchard, infected leaf and fruit samples were taken from examined sweet orange, mandarin, and lemon trees that were showing the typical symptom of the disease. Samples were placed in transparent plastic bags, covered with brown paper bags, labeled properly, kept in cool icebox containers, and then transported to the Laboratory for isolation, purification, and identification of the causative pathogen.

2.5. Isolation and Identification of the Pathogen

In the laboratory to disinfect the working environment and essential tools for the work, initially wiping of the work area with 70% ethyl alcohol and dipping of the laboratory instruments such as; Forceps, Scalpels, Needles, and Knives in 70% ethyl alcohol and flame drying of them were done frequently. The same procedure was also repetitively applied at the end of every laboratory session.

Then leaf and fruit samples were washed in tap water and surface sterilized in 70% ethanol each for one minute, and then rinsed three times with distilled water was carried out. Sterilized leaves and fruit peels were cut, and four to six-leaf discs and peel pieces of fruits were placed on each Petri dish containing potato dextrose agar (PDA) in three replicates and incubated at 25˚C ± 1˚C, under the light. After five days of incubation suspected fungal colonies were developed.

Then with these colonies, purification was done using hyphal tipping onto fresh PDA medium and incubated for four to seven days at 25˚C ± 1˚C and was allowed to sporulate. And the sporulated cultures were examined under binocular microscope supported with cell sense entry soft were for identification, based on morphological characters of somatic and reproductive structures including spores/conidia and spore-forming structures. On the observed characteristics, confirmation was made with those available in the manuals of Barnett and Hunters [18]. Then to preserve the pathogen for future use, the agar deep culture tube inoculation method was implemented. That is, the pathogen was delivered using a needle and stabbed deep down the center of 1% water agar. The inoculated culture tubes were kept in a refrigerator at 4˚C.

2.6. Pathogenicity Test

A pathogenicity test was carried out by implementing Koch's postulate. The test was conducted on young and apparently healthy detached leaves of the same species from which the pathogen was isolated. The detached leaves were washed in distilled water; surface sterilized with 70% ethanol and rinsed repeatedly with sterile distilled water. In each Petri dish containing water agar (1%), two sterilized leaves of each species were placed, by keeping the lower side up. Then inoculation of leaves was carried out by placing drops of conidial suspension, with a concentration of 10^5 to 10^6 conidia mL^{-1} [8] [15] by placing a drop of spore suspension on leaves. The Petri dishes were enclosed with Para film to maintain high relative humidity that can facilitate the infection and significantly increase the success rate of the test. Whereas, controls were maintained using only distilled water in place of inoculum suspension. Cultures were incubated at 26˚C for two weeks, and inoculated leaf samples were regularly observed for the appearance of disease symptoms.

Then the re-isolation procedure was carried out from newly inoculated leaves to demonstrate Koch's postulate. And the re-isolated cultures were examined for cultural and morphological comparisons with the original cultures to confirm that it is the same pathogen we have.

3. Result and Discussion

3.1. Distribution of Citrus Leaf and Fruit Spot Disease

The present two surveys result showed that in most of the studied areas leaf and fruit spot was the most prevalent disease of citrus. In both of the cropping seasons the same mean prevalence rate (80%) on leaves of sweet orange was computed. The highest disease prevalence (100%) was recorded in eight districts (Gondar Zuria, Derra, Mecha, Jabitehinan, Basoliben, Machakel, Ayehugugusa and Guangua). And it was null in two districts (West Dembia and Libokemkem) both in 2021 and 2022.

3.2. Severity and Incidence of Citrus Leaf and Fruit Spot Disease

This study result also revealed that in most of the surveyed areas (80%, or 8 out of 10 districts) highest average disease incidence (87.75%) and disease severity (24.43%) were recorded (**Figure 1**).

To indicate the association of *Pseudocoecospora angolensis* disease with agro-ecological factors, both in 2021 and 2022 correlation and regression analyses were carried out for both disease incidence and severity as response and altitude, average daily temperature, mean annual rainfall and tree age as predictor variables. And similar result was obtained. That is, linear correlation was detected only between mean annual rainfall and disease severity and incidence of leaves and fruits (**Table 1** and **Table 2**). As a result, regression analysis was conducted on these explained and predictor variables that showed linear correlation. And the regression analysis result indicated that during the two study years mean annual rain fall has a significant effect over disease severity and incidence of leaves and fruits at ($P \leq 0.05$) level.

3.3. Identified Agronomic and Pest Management Constraints

In the surveyed areas in both of the study years besides, citrus leaf and fruit spot

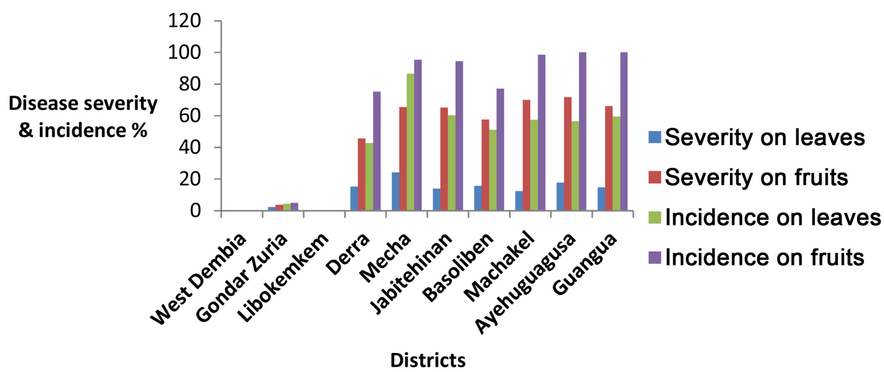

Figure 1. Severity and incidence of citrus leaf and fruit spot disease across districts.

Table 1. Pearson's linear correlation analysis of 2021.

Explained variables	Predictor variables			
	Altitude	Mean annual rainfall	Average daily temperature	Tree age
Severity on leaves	−0.08	0.71*	−0.17	0.04
Severity on fruits	−0.01	0.61*	−0.22	0.06
Incidence on leaves	−0.04	0.68*	−0.09	0.02
Incidence on fruits	0.0	0.62*	−0.19	0.04

*Correlation is significant at P ≤ 0.05 level.

Table 2. Pearson's linear correlation analysis of 2022.

Dependent variables	Independent r variables			
	Altitude	Mean annual rainfall	Average daily temperature	Tree age
Severity on leaves	−0.09	0.71*	−0.18	0.05
Severity on fruits	−0.01	0.60*	−0.25	0.03
Incidence on leaves	−0.05	0.69*	−0.09	0.02
Incidence on fruits	−0.09	0.62*	−0.12	0.03

*Correlation is significant at P ≤ 0.05 level.

disease high insect pest predominantly scales and leaf miners (100%) infestation were observed and caused a devastating effect both in citrus production.

However, to resolve the problem there was no insect pest management effort made in all assessed orchards (100%, or 20 out of 20). Besides, moisture stress as a result of absence or low irrigation schemes, lack of improved agronomic practices (pruning, weeding and clearing) and shortage of better quality citrus cultivars were contributed a lot to the existence of low citrus fruit productivity and production as well.

According to the information obtained from the interviewee, chemical fertilizer application was practiced only by the government-owned orchards (20%, or 4 out of 20). But it was not applied at the recommended rate and frequency. However, application of animal manure was practiced only by small-scale farmers' owned orchards (80%, or 16 out of 20).

3.4. Pure Isolates of *Pseudocercospora angolensis*

By implementing hyphal tipping method pure isolates of the pathogen was obtained (**Figure 2**).

3.5. Pathogenicity Test

To confirm the pathogenicity of the identified isolates of *Pseudocercospora angolensis* on its host, Koch's postulates were implemented. And the result showed that *Pseudocercospora angolensis has* the capacity to produce its typical disease symptom based on its availability. As a result, during 2021 among the tested isolates of the pathogen 81.25%, 66.66% and 41.66% of the inoculated sweet orange, mandarin and lemon leaves caused typical symptom of the disease (**Table 3**). Whereas, in 2022 the percentage of leaves caused typical symptom of the disease were 83.33%, 75% and 33.33% of inoculated sweet orange, mandarin and lemon leaves, respectively (**Table 3**). And the test isolates were reliably recovered from inoculated symptomatic leaf tissues. However, there was no symptom observed on water inoculated controls.

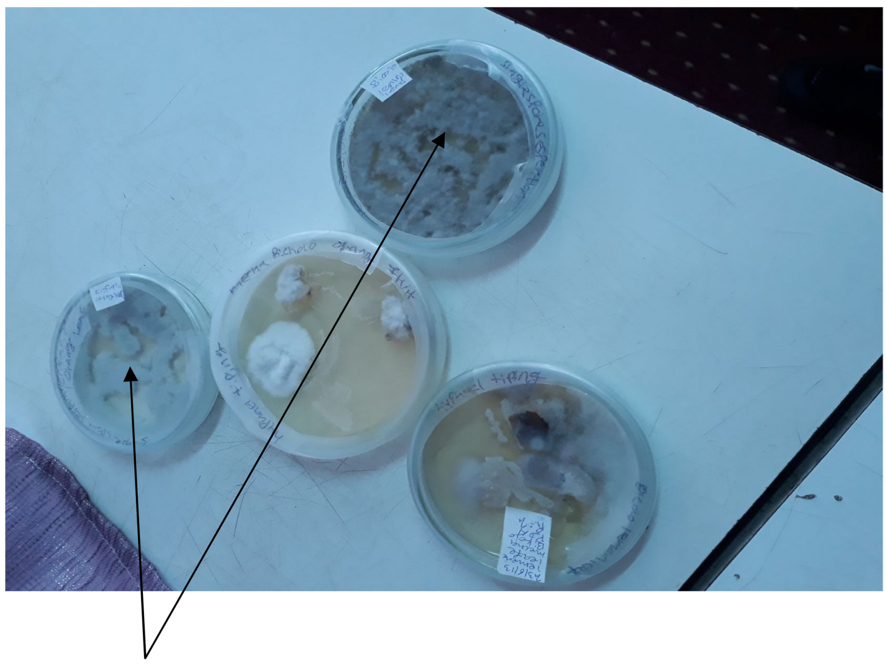

Figure 2. Pure isolates of *Pseudocercospora angolensis.*

Table 3. Inoculation success of conidial suspension of *Pseudocercospora angolensis.*

Citrus species	No of inoculated leaves		No of infected leaves		Infection percentage	
	2021	2022	2021	2022	2021	2022
Sweet orange	48	48	39	40	81.25	83.33
Mandarin	12	12	8	9	66.66	75
Lemon	12	12	5	4	41.66	33.33

4. Discussion

Visible variations among surveyed districts were observed in their computed disease prevalence, incidence and severity rates. These variations could be attributed to differences among districts in their received amount of mean annual rain fall. This is because, among agro-ecological factors that were under gone correlation analysis, linear correlation was detected only in between mean annual rainfall and disease incidence and severity. This result was consistent with the former report [17]. According to the report, the development and spread of *Pseudocercospora angolensis* is favored with high rain fall and humidity. However, the reason for the absence of the disease in two districts (West Dembia and Libokemkem) could be attributed to the absence of inoculum source that could be brought by wind and by transporting of infected fruits and propagating materials.

5. Conclusion

This study result showed that in most of the surveyed areas leaf and fruit spot was the most prevalent and long-lasting disease of citrus. And it is causing a devastating effect on citrus fruit production and productivity. As a result, most citrus growers were practiced uprooting of their citrus trees and replacing them by other fruit crops (Mango and Avocado).

6. Recommendation

Therefore, to provide sustainable citrus fruit productivity and production in areas where citrus leaf and fruit spot disease is predominant and ongoing, integrated and sustainable citrus leaf and fruit spot disease management practices should be implemented.

Moreover, in order to get full illustrates of the economic importance of the disease, it is useful to conduct similar disease surveys in major citrus growing areas of the country.

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Conflicts of Interest

The authors have no conflict of interest and all co-authors have seen and agree with the contents of the manuscript.

References

[1] Chung, K.R. and Timmer, L.W. (2009) Citrus Diseases Exotic to Florida: *Phaeoramularia* Fruit and Leaf Spot (PFLS). Fact Sheet PP-234. University of Florida, Gainesville. https://doi.org/10.32473/edis-pp155-2007

[2] Chalkley, D. (2012) Invasive Fungi: Angular Leaf Spot of Citrus-Pseudocercospora Angolensis. Systematic Mycology and Microbiology Laboratory, ARS, USDA,

Washington DC.

[3] Kuate, J. (1999) Fruit and Leaf Spot Disease Caused by *Phaeoramularia angolensis*: A Threat to Citrus Production. Research and Methods, FRuiTROP, No. 61.

[4] Seif, A.A and Hillocks, R.J. (1998) Some Factors Affecting Infection of Citrus by *Phaeoramularia angolensis*. *Journal of Phytopathology*, **146**, 385-391.

[5] De Carvalho, T. and Mendes, O. (1952) A Cercosporiosis Is Citrus. *Motambique*, **72**, 1-8.

[6] Kuate, J. (1998) Citrus Leaf and Fruit Spot Disease Caused by *Phaeoramularia angolensis* (Cercospariose des agrumes causee par *Phaeoramularia angolensis*). *Cahiers Agricultures*, **7**, 121-129.

[7] Eshetu, D. (1999) Occurrence, Prevalence and Control Methods of *Phaeoramularia* Leaf and Fruit Spot Diseases of Citrus in Ethiopia. *Journal of Fruits*, **54**, 225-232.

[8] Mohammed, Y. (2007) Distribution and Management of *Phaeoramularia* Leaf and Fruit Spot Disease of Citrus in Ethiopia. *Fruits*, **62**, 99-106. https://doi.org/10.1051/fruits:2007003

[9] Timmer, L.W., Garnsey, S.M. and Broadbent, P. (2003) Diseases of Citrus. In: Ploetz, R.C., Ed., *Diseases of Tropical Fruit Crops*, CABI, Walling Ford, 163-195. https://doi.org/10.1079/9780851993904.0163

[10] Yesuf, M. (2013) *Pseudocercospora* Leaf and Fruit Spot Disease of Citrus: Achievements and Challenges in the Citrus Industry: A Review. *Agricultural Sciences*, **4**, 324-328. https://doi.org/10.4236/as.2013.47046

[11] Amata, R.L., Otipa, M.J., Waiganjo, M., Wabule, M., Thuranira, E.G., Erbaugh, M., Chaube, H.S. and Singh, U.S. (1991) Plant Disease Management: Principles and Practices. CRS Press, Boca Raton, 319 p.

[12] Asmare, D., Derbew, B., Belayneh, A. and Mohammed, Y. (2014) Citrus Production, Constraints and Management Practices in Ethiopia: The Case of *Pseudocercospora* Leaf and Fruit Spot Disease. *Science*, *Technology and Arts Research Journal*, **3**, 4-18. https://doi.org/10.4314/star.v3i2.2

[13] Nwanosike, M.R., Mabagala, R.B. and Kusolwa, P.M. (2015) Disease Intensity and Distribution of *Exserohilum turcicum* Incitant of Northern Leaf Blight of Maize in Tanzania. *International Journal of Pure and Applied Biosciences*, **3**, 1-13.

[14] Amadi, J.E. (2008) Studies of the Etiology of Leaf Spot of Disease of Rough Lemon (*Citrus jambhiri*-B. Jamir; H. Jhambhiri). *Bio-Research*, **6**, 343-345. https://doi.org/10.4314/br.v6i1.28662

[15] Ezeibekwe, I.O. (2011) Study of Citrus Disease Prevalence on Four Citrus Varieties at the National Institute of Horticultural Research (NIHORT) Mbato, Okigwe, Imo State, Nigeria. *African Journal of Plant Science*, **5**, 360-364.

[16] Seif, A.A. and Hillocks, R.J. (1996) Epidemiology of Fruit and Leaf Spot of Citrus Caused by *Phaeoramularia angolensis* in Kenya: An Overview. *Proceedings of the International Society of Citriculture*, **1**, 338-339.

[17] Chaube, H.S. and Singh, U.S. (1991) Plant Disease Management: Principles and Practices. CRC Press, Boca Raton.

[18] Barnett, H.L. and Hunters, B. (1985) Illustrated Genera of Imperfect Fungi. 4th Edition, Macmillan Publishing Co., New York.