



# Prevalence and Assessment of Disease Incidence and Severity in Citrus Orchards of Sub Zoba Hamelmalo

## Autors & Affiliation

Amanuel Tesfagerish<sup>1</sup>,

Ariam Ghebrebrhan<sup>1</sup>,

Rahel Temesghen<sup>1</sup>,

Rodas Dawit<sup>1</sup>

Gangapuram Sethumadhava Rao<sup>1\*</sup>

<sup>1</sup>Department of Plant Protection,  
Hamelmalo Agricultural College, Keren

## \*Corresponding author:

Gangapuram Sethumadhava Rao

E-mail: [sethumadhava.g@gmail.com](mailto:sethumadhava.g@gmail.com)

Phone: 00291-0729394

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## Abstract

Citrus (*Citrus* spp. L.) production in Eritrea covers over 2409 ha of the land but the average potential of production is 91 q/ha to 150 -170 q/ha. This low yield might be due to lack of knowledge on management practices, adverse environmental factors and different diseases caused by various pathogens. Moreover, there is no proper recorded data on the identification of diseases on citrus and its intensity, hence, this survey: to identify the prevailed diseases; assessment of disease incidence, severity and calculate the 'Area Under Disease Progress Curve' (AUDPC). A survey was carried out in three orchards in each five citrus producing villages (*Fredarb*, *Tsebabo*, *Hamelmalo*, *Wazntet* and *Genfelom*) from sub-zoba Hamelmalo. The fungal diseases found on citrus were brown spot, sooty mold, gummosis, black spot, scab and melanose; the bacterial infection was canker; and viral diseases were leprosis and chlorotic dwarf. The highest percentage of disease incidence was recorded *Fredarb* with 77.16% and the lowest was found in *Genfelom* with 47.29 % in the same month. The highest percentage of diseases severity was found in *Hamelmalo* with 40.02%. On the other hand, the least percentage of disease severity was found in *Genfelom* with 25.04%. The influence of epidemiological factors showed that they could be some of the reasons to develop and spread the mean disease incidence (65.6%) in February, 2018. The AUDPC was recorded highest (2829) in the orchard 3 of *Hamelmalo*, and lowest (1152) in orchard 3 of *Tsebabo*. A decreasing trend of temporal disease data from the first month to the third month was estimated in the orchard 2 and orchard 3 of *Fredarb*, orchard 2 of *Genfelom*, orchard 1 of *Hamelmalo*, all orchards of *Tsebabo* and in both orchard1 and 3 of *Wazntet* villages.

**Keywords:** AUDPC; Disease Identification; Disease Incidence; Disease Severity; Environmental factors.

## Introduction

Citrus (*Citrus* spp. L.) fruit, such as oranges, lemons, grapefruit, and limes belong to family Rutaceae, have been cultivated in an ever-widening area since ancient times. Citrus originated from the parts of Southeast Asia, and today the crop is cultivated in wide areas mainly in tropical and sub-tropical regions of the world between 40° North & South latitude in over 137 countries. Brazil, China, the United States of America, India, Mexico and Spain are the world's largest citrus producing countries in the world<sup>1</sup>.

In the early 1960s, Eritrea used to export citrus fruits to the neighboring countries and Italy. Now, citrus is one of the main fruit crops cultivating in the lower and mid-altitudes of the country under irrigation. Citrus production in Eritrea covers over 2409 ha of land and the major citrus producing areas in the country are *Alla, Gashbarka, Ghindae, Anseba River, Barka, Gensel, Gundet or Mereb, Shambuko, Tesseneye* (river bank of Gash) and *Omhajer* (along the bank of river Setit/ Atbara). The main citrus types that are produced in Eritrea are orange, mandarin, grab fruit, lemon and lime. Orange is the dominant type that covers the largest area 2064 ha in the country; lemon and mandarin are produced in 397 and 45 ha in the country, respectively<sup>2</sup>. The average potential of producing is 91 q/ha to 150 -170 q/ha. Whereas the expected potential yield is 200 q/ha which is twice the production of the present cultivation practices and thus the low yield is due to many factors such as biotic, abiotic and socio-economic factors (*i.e.* no supply of pesticides, poor knowledge of management practices by the farmers).

Several species and citrus varieties are subjected to different diseases caused by fungi, bacteria, mycoplasma, viruses and phanerogamic parasites. In some others, nutrient deficiencies and

other environmental effects lead to different physiological disorders. Citrus fruits can commonly be affected by different diseases and pests and these can cause a great loss in the farmers' yield<sup>3</sup>. This temperate and sub temperate fruit crop is mostly cultivated under irrigation and they are available throughout the year in Eritrea. Due to inappropriate control practices such as, spacing, irrigation, application of fertilizers and an indiscriminate spray of pesticides can cause fruit droppings. In addition to these problems infestations caused by insect pests and also pathogen infections lead to a great economic loss to the farmers. It is therefore, this study with the objectives of identify the diseases, disease incidence, severity and to calculate the Area Under Disease Progress Curve (AUDPC) in three citrus orchards of each five villages of sub-Zoba Hamelmalo.

**Materials and Methods:**

**Site Description:**

Survey was carried out on occurrence of diseases, disease incidence and severity of citrus in sub-Zoba of Hamelmalo which is located in Zoba Anseba about 15 km north of Keren (Fig.1).



Fig. 1. Hamelmalo zone (dotted red) in Anseba region (circled) of The State of Eritrea (Source: MoA, 2000)<sup>4</sup>

Most of the agricultural lands are in valley areas and or on either side of the ‘Anseba River’. The other main rivers passing in the Sub Zoba are *Beyan*, *Andir* and *Gararb* which are seasonal type of rivers or streams. *Anseba River* is the biggest of all the rivers (Table 1).

**Table 1: Altitude, Temperature and Rainfall of sub-Zoba of Hamelmalo**

Altitude	Temperature	Rainfall and Water source
Average altitude is 1330m above mean sea level.	Ranges from 16°C to 38°C with the hottest month being May and the coldest month in January.	Ranges from 465 to 673mm with a mean annual rainfall of 537.6 mm. The rain starts in June and lasts until the end of September. July and August are the months with the highest rainfall.

Source: Anonymous, (1997)<sup>5</sup>.

**Data Collection**

During the survey, five main citrus producing locations or administrative villages (*Fredarb*, *Tsebabo*, *Hamelmalo*, *Wazntet* and *Genfelom*) from sub-zoba Hamelmalo and three sites from each village were selected at a distance of half a kilometer. In each site 10 percent citrus plants were selected at random for the assessment of diseases and their percentages. The samples i.e., infected leaves, fruits and stems were collected and identified based on visual symptoms and observed microscopically<sup>6</sup>.

**Isolation and Identification**

For the isolation of pathogens, Moist Blotter Paper Method and Potato Dextrose Agar Media were used<sup>7</sup>. Identification of fungi was carried out based on characterization of symptoms and examined under a compound microscope with 40X magnification power isolating the minute colony taken from infected areas of plant parts<sup>8-10</sup>.

**Data analysis**

For estimation of disease incidence on individual sampled branches, the whole branches

surface area was considered as 100% then the diseased area was estimated visually.

**Disease Incidence:** The following formula used for calculating the disease incidence (DI):

Percent Disease Incidence = Number of parts/plants infected/ Total number of parts/plants observed x 100.

**Disease Severity:** The disease severity (DS) was calculated, considering the “1-5” rating **Disease Index Scale** with the formula mentioned by Jagtap et.al. (2012)<sup>11</sup> (Table 2).

**Table 2. Disease Index Scale**

Scale	Percentage of Infection	Infection Rate in Branch	Infection Rate in Leaf
1	0-5%	No symptoms or small lesions appear	
2	5-25%	Top three leaves or twigs are diseased	
3	25-50%	Nearly half part of the branch or the plant is diseased	
4	50-75%	Upper three-quarter part of the plant is diseased	
5	75-100%	Nearly entire plant diseased or discolored	

Percent disease severity= Sum of all infected ratings/ Number of rating x Maximum rating scale x 100.

**Analysis of Disease Progress:** The trapezoidal integration method of an integral model, i.e., Area Under the Disease Progress Curve (AUDPC) was used for each orchard using the following formula<sup>12</sup>.

$$AUDPC = \sum_{i=1}^{n-1} 0.5 (x_{i+1} + x_i)(t_{i+1} - t_i)$$

where,  $X_i$  is the cumulative disease severity expressed as a proportion at the  $i^{\text{th}}$  observation;  $t_i$  is the time (days/weeks/months) at the  $i^{\text{th}}$  observation and  $n$  is the total number of observations. Since the duration of assessment was not the same for each epidemic, AUDPC values were standardized by dividing the values by the total duration ( $t_n - t_1$ ) of the epidemic<sup>13 and 14</sup>.

### Statistical Analysis:

Data on disease incidence, severity and AUDPC were subjected to analysis of variance using GENSTAT software-2012. Least Significant Difference (LSD 0.05) was employed to compare assessment means.

### Results and Discussion:

#### Identification of Diseases and Disorders

About nine diseases and some disorders were observed in fifteen orchards from five villages of sub-Zoba Hamelmalo. Majority of the diseases were caused by fungi and some were noticed bacterial and viral which were transmitted by insect pests. It is listed in Table 3, the fungal diseases found on citrus were *Alternaria* brown spot, black sooty mold, citrus gummosis, citrus black spot, citrus scab and melanose; the bacterial infection was citrus canker; and viral diseases were leprosis and citrus chlorotic dwarf (CCD) (Plates 1-5).

**Alternaria** disease symptoms were noticed in Genfelom and Hamelmalo villages which were in similar to the outcomes of Emperor Mandarin in Florida<sup>15</sup>, South Africa<sup>16</sup>, Israel<sup>17</sup>, Turkey<sup>18</sup>, Colombia<sup>19</sup>, and Spain<sup>20</sup>. The present results are also in agreement with Naqvi, (2014) that the leaves susceptible from formation to when fully expanded and hardened<sup>21</sup>.

**Black Soot Mold** was common in all villages and was observed on leaves fouled with honeydew defecated by sucking insects such as aphids, mealybugs, common soft scale insects - *Coccus viridis* (green coffee scale), *Ceroplastes rubens* (pink wax scale) and whiteflies. The fungal mycelium formed a black papery layer that can be peeled off from the underlying leaf. According to Lemos (2006)<sup>22</sup> and Nelson, (2008)<sup>23</sup>, the black coating causes a reduction of incident light by

physical hindrance, depending on the host species and the level of fungal growth.

**Gummosis** was noticed in five villages, is also known as Brown Rot Gummosis<sup>11</sup>. The symptoms were visible firstly as thinning out of the canopy. As the disease progresses, the foliage turns yellow and permanent dieback of twigs occur. Eventually, oozing of gum from the affected trunk and cracks in the bark. In the advanced stage, this disease leads to 'dieback of twigs' which was noticed in some orchards. This result is supported by Bertus, (2002)<sup>24</sup> and Ezeibekwe, (2011)<sup>25</sup> that the diseases of citrus are caused by various species of *Phytophthora* and these diseases include root rot, collar rot and brown rot of fruits. And also, these outputs are similar with the results of the survey done by Syed *et al.*, (2016), that citrus gummosis was found in three citrus species *viz.* orange, lemon and mandarin within the nine administrative regions of three sub zobas of Zoba Anesba<sup>26</sup>. In their research, the citrus crops showed highest/maximum disease incidence in Hagaz, the lowest/minimum incidence was found in Hamelmalo. It was also observed that the highest disease severity was noted in Hagaz region and the lowest was recorded in sub-region Elabered<sup>26</sup>.

**Citrus black spot** symptoms were recorded in the villages of *Fredarb*, *Genfelom* and *Hamelmalo*. This is caused by fungi, *Guignardia citricarpa*. Initially, red-brown and pin-pointed dots appeared later they develop into larger, circular necrotic lesions with gray centers and red or brown margins. On fruits superficial lesion appeared on the fruit rind. It typically begins as small orange or red spots with black margins and eventually becomes brown or black. Upon maturing, the mycelium grows into the outer rind, also known as a flavedo. It is in agreement with Agrios (2005), that the ascospores can infect fruit, they have not yet been observed developing on fruit<sup>27</sup>.

**Table 3: Diseases, Causal Organism and visible Symptoms of Various parts of the Citrus Plants in Five Villages of sub-Zoba Hamelmalo**

Village	Disease	Causal Organism	Symptoms noticed on leaves	Symptoms noticed on fruits and other parts of the plant
Genfelom and Hamelmalo	Alternaria Brown Spot (Fig. 2a-c)	<b>Fungi:</b> <i>Alternaria citri</i> and <i>A. alternata</i> pv. <i>citri</i>	Firstly, small brown spots that develop yellow halos on leaves. Later lesions expand into circular or irregular shapes. Badly affected leaves fall off.	Starting a small dark speck develops into large black corky eruptions on fruits. The eruptions can fall off leaving craters on the fruit surface.
Fredarb, Genfelom, Hamelmalo, Tsebabo and Wazntet	Black Soot Mold (Fig. 2d-e)	<b>Sooty mold:</b> <i>Capnodium</i> spp., epiphytic saprobes live off honeydew, the droppings of aphids and scale insects.	Blackish sooty mold develops on leaves fouled with honeydew excreted by sucking insects such as aphids, mealybugs, scales and whiteflies. The fungal mycelium forms a black papery layer that can be peeled off from the underlying leaf.	Sooty molds appear on the stems of plants as a superficial, black growth of mycelium forming a film or crust on these plant parts. Blackish sooty mold develops on fruit also.
Fredarb, Genfelom, Hamelmalo, Tsebabo and Wazntet	Citrus Gummosis (Fig. 2f-h)	<b>Fungus:</b> <i>Phytophthora citrophthora</i> produce large number of motile zoospores.	The fungus survives in the form of dormant mycelium and under moist conditions.	Gum formation on the trunk or branches, exudes from blisters containing gum pockets on the trunk. Infection starts as water-soaked patches on the basal portions of the stem. Bark dries, shrinks and cracks and shreds in lengthwise vertical strips.
Fredarb, Genfelom and Hamelmalo	Citrus Black Spot (Fig.2i)	<b>Fungi:</b> <i>Guignardia citricarpa</i> (Anamorph: <i>Phyllosticta citricarpa</i> )	Symptoms are visible on fruits as a superficial lesion on the fruit rind.	It typically begins as small orange or red spots with black margins and eventually becomes brown or black.
Genfelom, Hamelmalo, Tsebabo and Wazntet	Citrus scab (Fig. 2j-k)	<b>Fungus:</b> <i>Elsinoe fawcettii</i>	Small, semi-translucent dots like lesion develops on leaves. The opposite surface corresponding to the warty growth shows a circular depression with a pink to red center.	Lesions consist of corky projections, on the fruit, which often break into scab affecting larger areas on the fruits.
Hamelmalo, Tsebabo and Wazntet	Melanose (Fig.2 l-n)	<b>Fungus:</b> <i>Diaporthe citri</i>	The lesions on leaves begin as small, circular dark depressions with yellow margins. As the disease progresses, spots become raised and turn dark brown.	Spots initially are small on fruits, light brown, and sunken. Spots that are close together can coalesce to form a rough-irregular or rough surface.

Genfelom, Hamelmalo, Tsebabo and Wazntet	Leprosis (Fig.2o-q)	The <i>Citrus Leprosis Virus (CILV)</i> is a plant rhabdovirus: It is transmitted by the genus <i>Brevipalpus</i> mite.	Leprosis symptoms develop on leaves of citrus and sweet orange. These chlorotic symptoms become necrotic in the center. Leaves and fruit abscise and fruit will drop prematurely.	Leprosis symptoms develop in young twigs also. These lesions are first chlorotic and then become necrotic. Extensive lesions on twigs cause dieback. Fruit abscises and drops prematurely.
Genfelom, Hamelmalo, Tsebabo and Wazntet	Citrus Canker (Fig. 2r-s)	<b>Bacteria:</b> <i>Xanthomonas axonopodis</i> pv. <i>citri</i>	First raised, watery spots appear on leaves. Later spots become thickened, brown and corky. Infection to petioles, mid rib causes pre mature defoliation.	Initially raised, watery spots appear on twigs, thorns and brunches. Later spots become thickened, brown and corky. In severe attack symptoms produced on all plant parts.
Fredarb, Hamelmalo, Tsebabo and Wazntet	Citrus Chlorotic Dwarf (CCD) (Fig.2t-u)	<i>Citrus Chlorotic Dwarf (CCD) Virus</i>	A chlorosis and reduced leaf size were found on citrus hosts. Various chlorotic patterns, crinkling and other types of leaf distortions were induced in young leaves by this virus.	Not found on the other parts of plant.

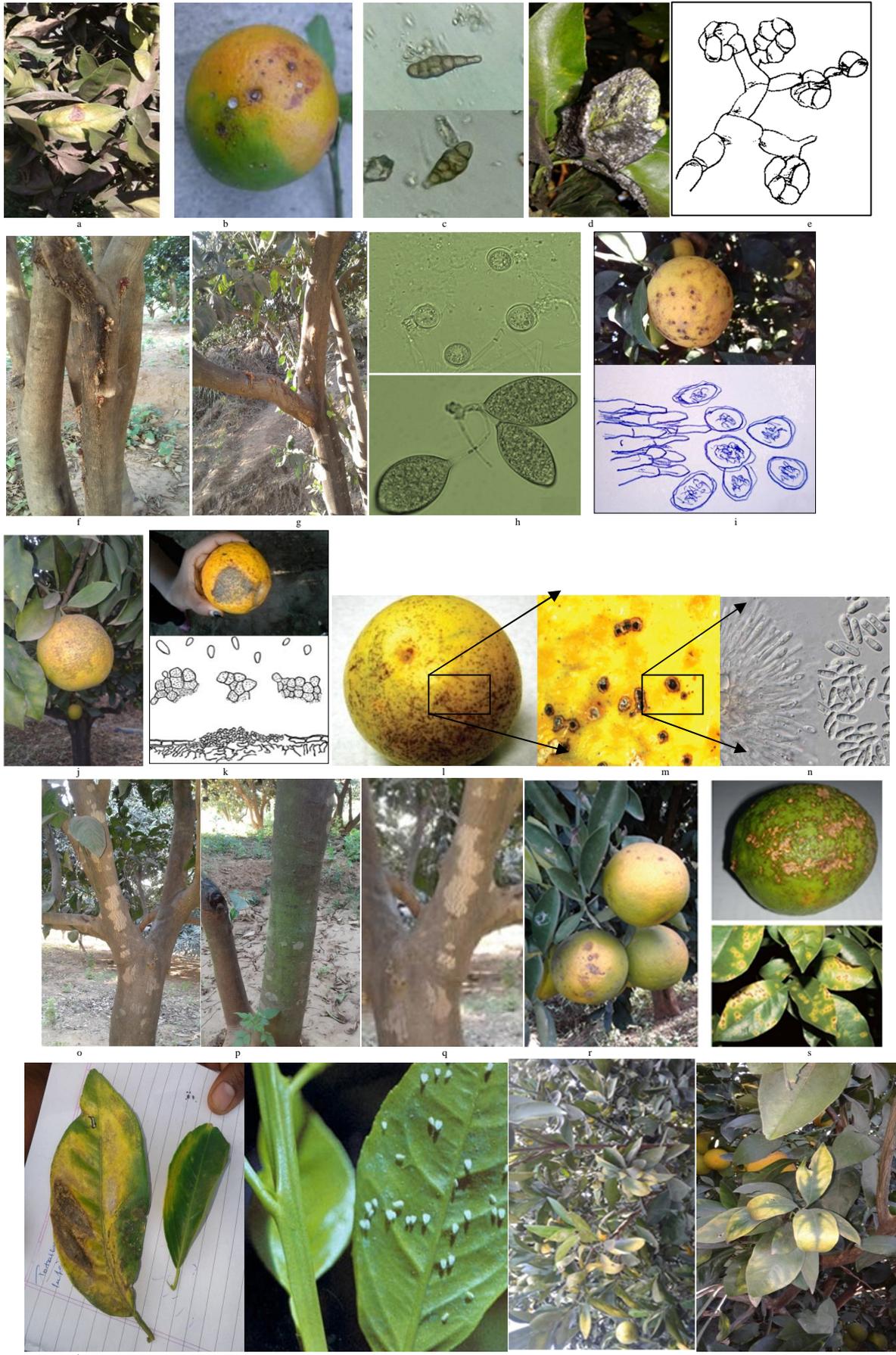


Fig. 2. Concentric symptoms of brown spots on leaves (a); fruits (b); fungal conidia of *Alternaria* spp. (c); and Black Soot raised kimps on leaves (c); and fungus *Capnodium* (e); Gum formation on trunk (f); on the branches (g); *Phytophthora citrophthora* antheridial attachment and papillated sporangia (h); Black spot disease on fruit; and *Guignardia citricarpa* (i); Scab disease on lemon fruit (j); fungus *Elsinoe fawcettii* (k); Fleck and Specks on fruits of Melanose disease (l-m); and *Diaporthe citri* (n); Leprosis, bark scaling on the tree trunk (o to q); Citrus canker symptoms on fruits; (r) and leaves (s); Crinkled leaf distortions (t); the whitefly (*Parabemisia myricae*) on citrus leaves (u); which transmit the virus to cause Citrus Chlorotic Dwarf (CCD); Yellowing on the leaf edges (v) while the centers remain green (w) due to Zinc or/and Magnesium deficiency.

**Citrus scab** was widespread in areas Genfelom, Hamelmalo, Tsebabo and Wazntet. The results of this survey were found to be at par with the findings Spósitoa *et al.*, (2011), who have explained that suitable conditions of temperature and rainfall or high humidity prevail the disease<sup>28</sup>. Fungal spores of *Elsinoe fawcettii* are readily produced on the surface of scab lesions on leaves and young fruits throughout the year<sup>29</sup>.

**Melanose** was observed in Hamelmalo, Tsebabo and Wazntet. It visible as raised, red to brown pustules on the leaves, twigs and fruit. These survey findings are in agreement with the outcomes such as lesions coalesce to form a mud cake symptom on fruit<sup>29</sup>. According to Whiteside, (1977a), the dead twigs decay rapidly in tropical, high-rainfall areas, and serve as a source of inoculum, similar symptoms were observed in this survey too<sup>15</sup>.

**Leprosis** is observed in Genfelom, Hamelmalo, Tsebabo and Wazntet villages. This disease with similar symptoms, also distributed in African countries such as Angola, Burundi, Cameroon, Egypt, Kenya, Malawi, Mauritania, Mauritius, Mozambique Réunion, Rwanda, South Africa, St. Helena, Sudan, Tanzania, Tunisia, Uganda, Zaire and Zimbabwe <sup>30</sup>.

**Citrus canker** was observed in four villages that was severe is also, in parallel with the exploration of Mohammadi *et al.*, (2001), that citrus

canker was extremely persistent when it becomes established<sup>31</sup>. However, during the spring and summer seasons, rains are combined with wind speeds in excess, damage from the disease can range from nominal to significant<sup>32</sup>.

The **Citrus Chlorotic Dwarf (CCD)** was noticed in Fredarb, Hamelmalo, Tsebabo and Wazntet villages on tender shoots. This disease was also found with similar symptoms in the Adana region of Turkey in 1986 and reached, in just a few years, epidemic levels in citrus nurseries. During the years of 1993-1994, 40% of 2,800 trees of grapefruit, mandarin, lemon and sweet orange were shown CCD symptoms<sup>33</sup>. The vector for CCD is a whitefly, *Parabemisia myricae* infests only the very young tender shoots of citrus and cannot penetrate on older leaves. Therefore, it is always found on new shoots. It is similarity with Korkmaz *et al.*, (1994), studies which revealed that although it can infest all citrus varieties it prefers those which have many flushes producing tender leaves such as lemon and grapefruit<sup>34</sup>.

**Disorders:**

Almost in all villages different types of disorders were found (Table 4). It is due to the deficiency of Zinc, Magnesium and paucity of nutrients in the soil that show defect of citrus plants. Zinc and Magnesium deficiency showed yellowing on leaves (Fig. 2v-w). It is in line with Satyagopal *et al.*, (2014) that lack of minerals such as Zn and Mg, leaves turned into yellow<sup>35</sup>.

**Table-4. Types of Disorders in Five Villages of sub-Zoba Hamelmalo**

Villages/Orchards		Disorders
Fredarb	Orchard 1	Leaf deformation, stunting
	Orchard 2	Yellowing of leaves
	Orchard 3	No disease has been found
Genfelom	Orchard 1	Leaf deformation, zinc deficiency
	Orchard 2	Stem discoloration, magnesium or deficiency
	Orchard 3	Leaf discoloration, zinc or magnesium deficiency,
Hamelmalo	Orchard 1	Stem discoloration and stunting
	Orchard 2	Leaf discoloration and fruit deformation, zinc or magnesium deficiency
	Orchard 3	Leaf discoloration and fruit deformation, stunting, zinc or magnesium deficiency
Tsebabo	Orchard 1	Stem discoloration, zinc or magnesium deficiency
	Orchard 2	Fruit deformation, magnesium or zinc deficiency
	Orchard 3	Stunting and leaf deformation
Wazntet	Orchard 1	Leaf discoloration, stunting, Zn or Mg deficiency
	Orchard 2	zinc or magnesium deficiency
	Orchard 3	No disease has been found

**Assessment of Diseases:****Assessment of Disease Incidence in Sub-Zoba Hamelmalo:**

The survey was done on randomly selected three citrus orchards from five villages of Sub-zoba Hamelmalo within three months at one-month interval. In this research, all diseases were taken into consideration to calculate the disease incidence. The highest percentage of diseases incidence was recorded in Fredarb with 77.16%. On the other hand, the lowest percentage of disease incidence was found in Genfelom with 47.29%. However, it is observed that the mean value shows there was a statistically significant difference in December in Hamelmalo among the other villages (Table 5).

The maximum percentage of disease incidence was found in Hamelmalo (88.9%) that might be due to poor management practices. And also, it is noticed that the farmers do not have awareness about the different citrus diseases and their development on the citrus plant. According to Gopal et al., (2014) that there is plethora of constraints for citrus cultivation viz., non-availability of disease-free planting material, transmissible diseases and insect pests, general

neglect, poor management practices and cultivation in unsuitable soils<sup>29</sup>.

It is indicated that there was statistically significant different in December and January, this may be due to less spacing among plants which is not more than 3 meters that favors fungal and root pathogens as citrus trees do not need high moistness, especially during blooming season and excess water is bad for their growth. Besides that, there was high relative humidity (74.2%) was observed in January in lowest relative humidity (62.6%) was recorded in February (Fig.2). These results in agreement with Fawcett (1936)<sup>36</sup>, Fraser (1949)<sup>37</sup>, Govinda (1954)<sup>38</sup>, Klotz (1950)<sup>39</sup>, Ramakrishnan (1954)<sup>40</sup>, and Cheema *et al.* (1954)<sup>41</sup> unanimously agreed that environmental conditions influence the advance of the disease of gummosis to a great extent.

The influence of epidemiological factors such as the maximum temperature (29.86°C) and minimum temperature (10.71°C); and wind speed (4.35 m/s) showed that they could be some of the reasons to develop and spread the mean disease incidence (65.6%) in February, 2018.

**Table 5. Percentage of Disease Incidence of Three Orchards of Five Villages of Hamelmalo**

Villages	Orchards	December, 2017	January, 2018	February, 2018	Average of months	Average of orchards
<i>Fredarb</i>	Orchard 1	75.2	76.7	85.3	79.07	77.16
	Orchard 2	78.1	68.5	82.5	76.37	
	Orchard 3	84.9	82.6	60.6	76.03	
<i>Genfelom</i>	Orchard 1	37.2	34.6	85.3	52.37	47.29
	Orchard 2	33.5	36.2	82.5	50.73	
	Orchard 3	24.7	31	60.6	38.77	
<i>Hamelmalo</i>	Orchard 1	88.9	85.6	72.3	82.27	65.02
	Orchard 2	82.1	59	62.6	67.90	
	Orchard 3	51.8	51.2	31.7	44.90	
<i>Tsebabo</i>	Orchard 1	81.9	75.8	70.4	76.03	72.45
	Orchard 2	82.6	79.8	51.9	71.43	
	Orchard 3	65.7	74.6	69.4	69.90	
<i>Wazntet</i>	Orchard 1	41.6	65.1	32.5	46.40	56.05
	Orchard 2	61.4	68.6	81.7	70.57	
	Orchard 3	62.6	54.9	36.1	51.20	
<b>MEAN</b>		<b>59.8</b>	<b>63.2</b>	<b>65.6</b>		
<b>*LSD at 5%</b>		<b>S</b>	<b>S</b>	<b>NS</b>		
<b>CV</b>		<b>28</b>	<b>14.3</b>	<b>25.1</b>		
Temp. maximum (°C)		29.3	28.17	29.86	29.67	

Temp. minimum (°C)	8.83	4.92	10.71	8.13	
Relative Humidity (%)	69.6	74.2	62.6	68.8	
Wind Speed (m/s)	4.45	3.9	4.35	4.23	

\* S= significant; NS=non-significant.

According to Chowdhury (1955a), the relative humidity and rainfall play a very important role in the development of scab than by temperature<sup>42</sup>. According to Aiyappa (1958) all cultivated varieties of citrus are susceptible to canker possibly due to heavy rainfall, high humidity and low temperature<sup>43</sup>. This survey also revealed that the highest percentage of relative humidity (74.2%) and minimum temperature (4.92°C) in January, 2018 could be a reason of *Xanthomonas* infection.

#### **Assessment of Disease Severity in Sub-Zoba Hamelmalo:**

All the surveyed diseases were taken into consideration to compute the disease severity. The highest percentage of diseases severity was found in Hamelmalo with 40.02%. Conversely, the lowest percentage of disease severity was found in

Genfelom village with 25.05%. During the survey, it was observed that the mean values showed there was statistically significant difference in December and January in Hamelmalo among the other villages. Over all, severity of the diseases from the analysis the statistical data revealed that the average disease severity was highest in Hamelmalo in December (44.1 %) and the lowest recorded was found in Tsebabo in February (18.6 %) (Table 6).

It is documented that there was statistically significant in January and February. Percentages of relative humidity 69.6% and 74.2% were might be played a role in dispersion of inoculum to cause mean disease severity in December, 2017 (35.4%) and January, 2018 (29.1%), respectively. Another environmental factor, wind-speed (4.45m/s) in December, 2017 also one of the reasons to accelerate more disease severity in Hamelmalo.

**Table 6. Percentage of Disease Severity of Three Orchards of Five Villages of Hamelmalo**

Villages	Orchards	December, 2017	January, 2018	February, 2018	Average of months	Average of orchards
<i>Fredarb</i>	Orchard 1	29.5	35.4	29.8	31.57	29.18
	Orchard 2	39.8	20.1	21.4	27.10	
	Orchard 3	39.8	32.7	14.1	28.87	
<i>Genfelom</i>	Orchard 1	27.5	25.4	29.8	27.57	25.04
	Orchard 2	28.7	30.9	21.4	27.00	
	Orchard 3	20.6	27.5	13.6	20.57	
<i>Hamelmalo</i>	Orchard 1	49.2	49.1	35.9	44.73	40.02
	Orchard 2	44.8	17.6	31.7	31.37	
	Orchard 3	38.2	56.7	37	43.97	
<i>Tsebabo</i>	Orchard 1	36	30.5	25	30.50	25.14
	Orchard 2	34.9	27.3	13.3	25.17	
	Orchard 3	24.2	17.5	17.6	19.77	
<i>Wazntet</i>	Orchard 1	31.4	18.4	14.7	21.50	29.15
	Orchard 2	40.1	30.6	49	39.90	
	Orchard 3	45.9	16.2	16.1	26.07	
<b>MEAN</b>		<b>35.4</b>	<b>29.1</b>	<b>24.7</b>		
<b>LSD at 5%</b>		<b>NS</b>	<b>S</b>	<b>S</b>		
<b>CV</b>		<b>17</b>	<b>38</b>	<b>42.4</b>		
Temp. maximum (°C)		29.3	28.17	29.86	29.1	

Temp. minimum (°C)	8.83	9.52	10.71	8.13	
Relative Humidity (%)	69.6	74.2	62.6	68.8	
Wind Speed (m/s)	4.45	3.9	4.35	4.23	

\* S= significant; NS=non-significant.

In addition to this, there may be biotic (pathogens, insect and etc.) and abiotic (relative humidity, temperature and wind speed) factors playing major roles in disease development. The noticed differences in the incidence and severity of diseases among the varieties could have been due to different resistant capabilities in the citrus species. This is in line with the work of Wutscher, (1998), who worked on varietal reactions of citrus, which showed varietal resistance and also proved that, fungi inoculated into the seedlings were more pathogenic or caused more extensive infections on citrus previously infected or weakened by an agent than on healthy plants<sup>44</sup>.

***Assessment of Disease Severity by AUDPC (Area Under Disease Progress Curve)***

The Area Under Disease Progress Curve (AUDPC) was calculated by trapezoidal integration

of the disease percentage values taken at different time-points (at monthly intervals) in three orchards from five villages and the disease severity ratings were recorded by using a 1–5 grade scale. The AUDPC was recorded highest (2829) in the Orchard 3, higher (2749.5) in Orchard 1 of Hamelmalo, then 2254.5 in Orchard 3 of Wazntet village. On the other side, it was lower (1338) in the Orchard 3 of Genfelom and lowest (1152) in Orchard 5 of Tsebabo village. It can be compared these surveyed results by measuring AUDPC, accounts for onset and rate of epidemic development. A decreasing trend of temporal disease data from first month to the third month was estimated in the orchard 2 and orchard 3 of Fredarb village, orchard 2 of Genfelom, orchard 1 of Hamelmalo, all orchards of Tsebabo and in both orchard 1 and 3 of Wazntet villages (Fig. 3). As pointed out by Campbell (1998), AUDPC shows the level of disease that induces stress during the season and can be used as predictor of yield<sup>45</sup>.

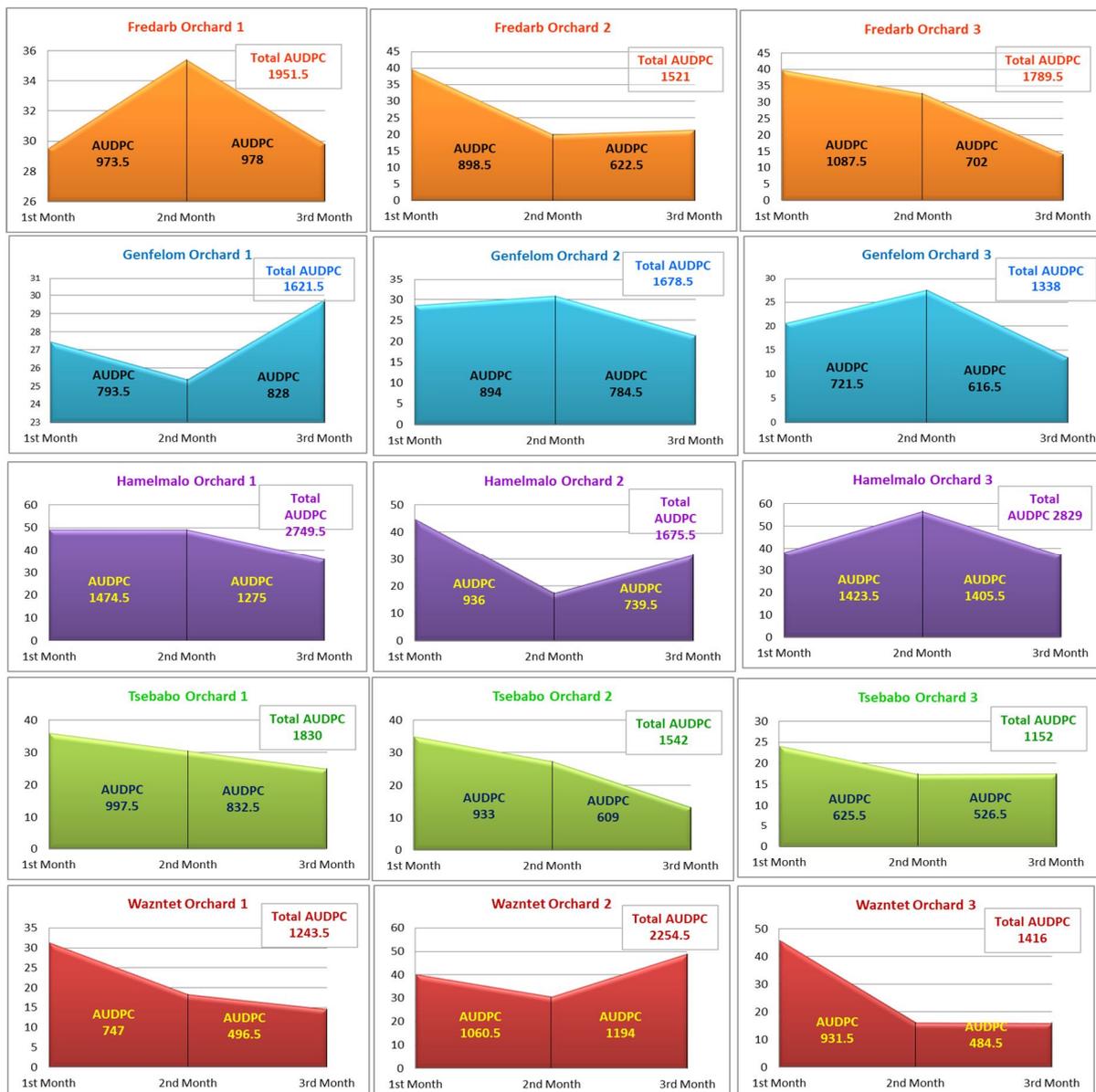


Fig 3. Standardized Area Under Disease Progress Curve (AUDPC) for Disease Severity in three Orchards of Five villages of Sub-Zoba Hamelmalo during the three Months Observation

**Application of Research:**

This survey clearly showed that the disease intensities, AUDPC and the influence of environmental factors on infections of citrus orchards in sub-zoba Hamelmalo can be predicted the disease warning systems. It can also be done to provide appropriate management practices to the farmers through extension and coordination with the help of the Ministry of Agriculture.

**Authors' Contributions:**

All the members were participated in both data collection and surveys. Moreover, data

compilation and supervision of the project were done by Dr. G. Sethumadhava Rao.

**Competing Interests:**

Authors have declared that no competing interests exist.

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