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# Ecosystem Monitoring on Leaves of Leaf Rust Disease of Maize (*Zea mays* L.)

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## Abstract

Endemic leaf rust disease always occurs in almost all maize plantations in Indonesia. Furthermore, the development of this disease differs concurrently and is greatly influenced by the ecological conditions of maize cultivation. Therefore, this study fills the epidemiological gap of diseases that has not been conducted against the epidemiology of maize rust. This identifies the causes of leaf rust that attacked the maize plants in two locations, namely Bayur and Muang Dalam, Lempake, Samarinda, Indonesia. This study also analyzed the relationship or model between ecological factors of temperature, humidity, and soil fertility on the intensity of leaf rust and the infection rate of maize leaf rust. Measurement of disease intensity, the rate at which it developed, soil fertility and temperature and humidity of the area are conducted in this study. Meanwhile, soil fertility also influenced disease progression and the nutrient-poor soils in two sites cause leaf rust disease to develop well. The identification results showed that the cause of maize leaf rust was *Puccinia sorghi* Schw. Therefore, the temperature accompanied by the increased humidity is directly proportional to the development of the leaf rust.

**Keywords:** maize leaf disease identification; plant disease epidemiology; *Puccinia sorghi* Schw; relative humidity; temperature

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## INTRODUCTION

Maize leaf rust is an airborne disease because its transmission mode is through the air and grows profusely when the pathogens encounter their host, such as maize. The pathogen penetrates the leaf, infects the plant and multiplies on the leaves (Surtikanti, 2011). The development of this disease is strongly influenced by its ecological conditions, including the temperature and humidity factors, as well as a plant supporting factor, namely soil fertility (Dhami et al., 2015). It is observed that 100% relative humidity and a temperature of 25 to 30$^{o}$C sporulation of Gray Leaf Spot (GLS) on maize are high, but the number and expansion of lesions are not significantly different at temperature > 25$^{o}$C. GLS develops slowly when the average daily temperature falls below 20$^{o}$C. In summer maize, the disease incidence is relatively high in the mountains and the valleys, while it is very low in the terai. However, the disease has attacked the winter and spring maize in the Terai of Nepal (Subedi, 2015).

Agricultural ecosystems are simpler and less stable, making them prone to disease development (Tilman et al., 2002). The stability of an ecosystem is determined by the diversity of structures and the characteristics of its components. Furthermore, this is achieved when an understandable and controllable interaction

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exists between components (Robertson et al., 2014).

Agroecosystems are dynamic, constantly changing in time and place, and are highly sensitive to influences within and outside the ecosystem. Therefore, to achieve ecosystem management goals, information about its state and dynamics obtained through monitoring activities is required. Furthermore, these activities are conducted to gain information about the state of the ecosystem, including weather, water, soil, pest and disease populations, natural enemies, crop damage and plant growth (Médiène et al., 2011).

According to statistical data, maize production in East Kalimantan increased in 2015, reaching 112,522 tons from the previous year (BPS-Statistics of Kalimantan Timur Province, 2016). Meanwhile, this has occurred due to an increase in productivity of 39.35%. Also, according to the field data, one of the constraints of maize production is the attack of leaf rust disease (Kusyanto and Hasmara, 2017).

Leaf rust is a disease that attacks maize plants caused by the fungus *Puccinia sorghi* Schw (Soenartiningsih et al., 2013). The factors that influence leaf rust disease are abiotic and biotic environmental factors such as climate as well as pests, respectively. Asynchronous climate change increases the development rate of fungus, inhibiting the growth of the maize plant itself. Therefore, it is necessary to control the leaf rust disease (Burhanuddin, 2015).

The agroecological approach strives to improve crop yields simultaneously while also understanding the processes that permit their maintenance (Wezel et al., 2014). However, the primary goal is to determine the long-term sustainability of agricultural systems. The primary foundation of agroecology is the ecosystem concept, defined as a functional system of complementary relationships between living organisms and their environment, delimited by arbitrarily chosen boundaries in space and time and appears to maintain a steady yet dynamic equilibrium (Gliessman, 1995; Ponisio and Ehrlich, 2016). In addition, Sopialena (2018) stated that the environment is one of the dominant factors in disease and its progression. Therefore, the research on ecosystems contributes to the development of sustainable agricultural systems (Perfecto and Vandermeer, 2015). The emergence of a disease requires at least three factors, such as the host plant, pathogen and environmental factors (Wakman and Burhanuddin, 2010).

Research on temperature and light on maize conducted by Negeri et al. (2013) showed that the maize phenotype was strongly influenced by temperature. Plants have hypersensitivity symptoms as a result of low temperature. Hence, there is an interaction between temperature and maize genetics. The hypersensitive reaction of maize controlled by the resistance genes is suppressed when maize is grown at a temperature greater > 30$^{o}$C. Therefore, the phenotype of maize is influenced by an increase or decrease in temperature.

To date, no studies have been conducted on maize's epidemiological leaf rust disease. Therefore, the importance of this study is to fill the gap in knowledge of diseases epidemiology on maize to identify the causes of leaf rust that attack in two research locations, namely Bayur and Muang Dalam, Lempake, Samarinda, Indonesia. Also, to examine the relationship or model between ecological factors (temperature, humidity and soil fertility) and the intensity of leaf rust disease as well as its infection rate. Furthermore, the calculation of the ecosystem monitoring model and damage level was performed as a measurement reference used as an early warning system in the initial control steps.

## MATERIALS AND METHOD

### Location of the research

This study was conducted on dry land maize plants in Bayur (latitude of -0.4736368 and longitude of 117.1645419) and Muang Dalam Village (latitude of -0.408272 and longitude of 117.1645419), Lempake, Samarinda, East Kalimantan, Indonesia. Also, at Laboratory of Plant Pests and Diseases, Faculty of Agriculture, Universitas Mulawarman. In addition, soil fertility was analyzed at Laboratory of Soil Science, Faculty of Agriculture, Universitas Mulawarman.

### Research design

*Survey*

A survey was conducted from January to April 2019 to determine the sampling sites. The first location was a maize field in Bayur Village, Lempake, which had previously been planted with long beans and had 144 maize plants (Bonanza F1 varieties). Meanwhile, the fertilizers and pesticides used for maize crops are NPK as well as Basmilang, respectively. The second sampling location was a maize field in Muang Dalam, Lempake, with a population of 100 maize plants (Bonanza F1 varieties) and had previously been planted with paddy. The fertilizers used included NPK, and the pesticides are Toxafine and Gramoxone. The sites' conditions are shown in Figure 1, and the distance between Bayur and Muang Dalam Village is 6 km.

a. b.

Figure 1. Maize plantation in Bayur (a), Muang Dalam (b)

This disease was identified in the laboratory, where pustules from infected leaves were scrapped using a needle sprayed with alcohol. After which, there were placed on an object glass and given a drop of methylene blue and safranin. The morphology of the pathogen was observed using an optical microscope.

*Observation parameters*

Using a wet-dry ball thermometer, the temperature and humidity were measured daily at the two locations starting from 1st week after planting. The data were collected three times a day, including morning 6 AM, afternoon 12 AM and evening 6 PM. Furthermore, the pathogen was identified in the Laboratory of Plant Protection, Faculty of Agriculture, Universitas Mulawarman by scraping the pustule on the surface of the maize leaf using an ooze needle. Afterward, there were placed on a glass object and observed under the microscope. The soil nutrient and pH analysis were conducted in the Laboratory of Soil Science,
Faculty of Agriculture, Universitas Mulawarman. The method used is total N (Khejdhal Method), potential-P (Extraction of HCl 25% using Bray or Olsen methods), available-K (1 N $NH_4OAc$ pH7) and soil pH (Electrometric Method using pH meter Hanna type HI 8424, by comparison, the liquid 1 : 2.5).

**Data analysis**

*Disease intensity*

The following formula is used to calculate the disease intensity (Equation 1).

$$I = \frac{\sum (ni \text{ x } vi)}{Z \text{ x } N} \text{ x } 100\% \quad (1)$$

Where; I represent the disease intensity (%), ni represent the numbers of plant leaves attacked, vi represent the scale of the attack, N represent the total number of leaves observed and Z represent the highest scale of attack categories

*Infection rate*

According to Jeffers (1965), the following formula is used to calculate the disease infection rate (Equation 2).

$$r = \frac{2.3}{t_2 - t_1}\left(\log_{10}\frac{x_2}{1 - x_2} - \log_{10}\frac{x_1}{1 - x_1}\right) \quad (2)$$

Where; r represent the infection rate, 2.3 represent the number of natural logarithmic conversion results to ordinary logarithm (Lnx = 2.3 Logx), t represent observation time interval, $x_2$ represent the proportion of diseased leaves at t, $x_2$ represent the proportion of diseased leaves at baseline.

Table 1. Scale category of leaf rust disease (Jeffers, 1965)

| Scale | Attack of the leaf area |
|---|---|
| 1 | 1 – 5% |
| 3 | 5 – 11% |
| 5 | > 11 – ≤ 25% |
| 7 | > 25 – ≤ 75% |
| 9 | > 75 – 100% |

Table 2. Infection rate criteria adopted from (Jeffers, 1965)

| Infection rate (unit of the week) | Criteria |
|---|---|
| < 0.11 | Mild |
| > 0.11 – < 0.50 | Moderate |
| > 0.50 | Severe |

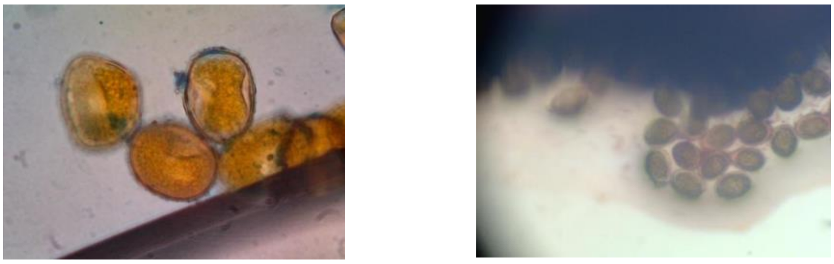
## RESULTS AND DISCUSSION

### Fungal identification

*P. sorghi* Scwh often appeared after silking in maize, having an early symptom of chlorotic specks on the leaf. Meanwhile, the obvious sign of this pathogen was golden-brown pustules or bumps on the above-ground surface of the plant tissue. These bumps were uredospores that could spread to other plants and cause further infection. Also, they were circular and powdery due to spores breaking through the leaf surface. The bumps are only about 1 to 2 mm each and are numerous with equal frequencies on upper and lower leaf surfaces (Burhanuddin, 2015; Dey et al., 2015).

Figure 2 shows details of *P. sorghi* Scwh based on the laboratory analysis of maize rust on leaves from two areas, Bayur and Muang Dalam plantations preferring humidity > 75% and temperature > 20$^o$C. The spores were unable to live without their host, hence, these are termed obligate parasites. First, *P. sorghi* scrubbed the pustules on the surface of maize leaves with a needle placed them on the object-glass, after which methylene blue and safranin staining was added and lastly viewed directly under a microscope.

Observations under a microscope with a 400x enlargement showed oval and round shapes. This is in accordance with Jeffers (1965), stating that the *P. sorghi* Scwh uredospore was yellowish to golden colour. The walls of the spores thickened it at both ends to golden. Also, its walls are thick with 4 to 5 holes-equator. Teliospore is brown, smooth, elliptical and round at both ends.



a. b.

Figure 2. Microscopic *P. sorghi* Schw methylene blue staining (a), safranin staining (400x) (b)

Fungal spores that spread through the air landed on the surface of healthy leaves. Although they were observed 11 times, leaf rust occurred at 55 days or 9th week after inoculation, as shown in the Table 3.

The early pustules grew gradually and only existed in the margins of maize leaves, as shown in Figure 3. Pustules were circular to round and orange to brown, and they rarely grow under the surface of maize leaves. Spores spread throughout the surface of maize leaves during their harvested period. RPK (1972) and Dey et al. (2015) stated that the leaf rust disease on maize caused by the fungus *P. sorghi* Schw occurred every growing season. Rust pustules usually occur in high relative humidity and high-temperature conditions, with characteristic chlorotic specks on the leaf surface. These soon developed into powdery, brick-red pustules as the spores broke through the leaf surface. The pustules were oval or elongated, about 1/8 inch long and scattered sparsely or clustered together. The leaf tissue around it was likely to be yellow or die, leaving lesions of dead tissue forming a band across the leaf, resulting in their death when severely infected. According to the age of the pustules, the red spores turned black and continued to erupt through the leaf surface. Some parts which are also prone to infection are the husks, leaf sheaths and stalks (Adegbite, 2011; Suriani et al., 2019).

Table 3. The observation results on leaf rust distribution in Bayur and Muang Dalam fields

| Observation week to | Number of pustules | |
|---|---|---|
| | Bayur | Muang Dalam |
| 1 | - | - |
| 2 | - | - |
| 3 | - | - |
| 4 | - | - |
| 5 | - | - |
| 6 | - | - |
| 7 | - | - |
| 8 | - | - |
| 9 | 149 | 276 |
| 10 | 2,413 | 3,655 |
| 11 | 11,151 | 9,361 |

**Disease intensity**

Disease occurrence is the proportion of individual hosts or organs affected by the disease regardless of how severe it might be, while its severity is the proportion of infected host surfaces to the total observed host surface, having symptoms expressed as a wide percentage to leaf surface area. Furthermore, this also refers to disease intensity (Agrios, 2005).

It has been observed that the average disease intensity ranged from 27 to 81% (Table 3), from moderate to severe criteria. The disease attacked the lower leaves until they went up, leaving the younger ones on the top. This was due to the disease's preference for humid and shady environments. Plant diseases were scored (assessed) by comparing plant symptoms to score tables from the Directorate of Protection. (Table 1).

**The infection rate of leaf rust disease**

The infection rate was obtained based on the proportion of diseased plants (percentage of disease), calculated every week according to the progression. The calculation of disease infection rates in the two research sites is shown in Table 4. Since the average rate of rust infection between the two places was not different, it implies that rust has been endemic in the area.

a. b.

Figure 3. Early pustules appear (a), pustules spread throughout the leaf surface (b)

Table 4. Results for calculation of Bayur infection rates and Muang Dalam infection rate

| Place | Infection rate (unit week) | | Mean average | Mean average temperature ($^{o}$C) | Mean average RH (%) |
|---|---|---|---|---|---|
| | r1 | r2 | | | |
| Bayur | 2.887 | 1.953 | 0.739 | 29.12 | 70 |
| Muang Dalam | 2.160 | 1.424 | 1.792 | 29.39 | 70 |
| Mean average | 2.523 | 1.688 | 1.081 | | |

Note: r1 = infection rate 1; r2 = infection rate 2; RH = relatif humidity

The observations are taken from 24 and 20 samples of plant leave in Bayur and Muang Dalam fields, respectively, which appeared to be increasing due to environmental conditions, especially temperature and humidity. The disease's development is also aided by significant rainfall, which causes infection to increase over time (Kinyua et al., 2011; Sopialena and Palupi, 2017).

The average infection rate in both places was more than 0.50 units week$^{-1}$ due to the rapid spread of spores. The attacks by the fungus of *P. sorghi* were too aggressive. Host varieties that are not resistant to disease and environmental factors support the development of pathogens. According to Jeffers (1965), the infection rate is used to identify the vulnerability of aggressive organisms, as well as the conducive [redacted]

expansion of lesions were not significantly different with > 25$^{o}$C temperature. Disease intensity occurred more in warm and humid conditions (Rahayu et al., 2018; 2020; Rochi et al., 2018). For example, rust disease was slow to develop when the mean daily temperature dropped below 20$^{o}$C (Sucher et al., 2017).

**Observation of thse spread area**

Based on the study results, pustules in leaf rust appeared in the 9th week, increasing weekly. Firstly, the pustule was visible on [redacted]

| Location | Base cation (pH 7) | | | | CEC | Base saturation (%) |
|---|---|---|---|---|---|---|
| | $Ca^{++}$ me 100g$^{-1}$ | $Ca^{++}$ me 100g$^{-1}$ | $Ca^{++}$ me 100g$^{-1}$ | $Ca^{++}$ me 100g$^{-1}$ | | |
| Bayur | 5.4666 | 4.12 | 0.39 | 0.3411 | 15.06 | 66.17 |
| Muang Dalam | 6.8700 | 3.88 | 0.85 | 0.4731 | 26.40 | 45.75 |

Table 5 shows that Bayur with pH 5.01 was included in the acid category. Total-N of 0.44%, potential-P and available-K was 40.37 ppm included in the high category and 171.50 ppm included in the very high category. $Ca^{++}$ 5.4666 ppm was classified as moderate, $Mg^{++}$ 4.12 ppm was classified as high, $K^{+}$ 0.39 ppm was categorized as low, $Na^{+}$ 0.3411 was classified as low, CEC 15.60 was classified as moderate and base saturation was 66.17% included in the high category.

In Muang Dalam, pH 5.44 was included in the acid category. Total-N of 0.33%, potential-P and available-K was l35.19 ppm included in the high category and 261.09 ppm included in the very high category. $Ca^{++}$ 6.87 ppm was classified as moderate, $Mg^{++}$ 3.88 ppm was classified as high, $K^{+}$ 0.85 ppm was categorized as low, $Na^+$ 0.4731 was classified as moderate, CEC 26.40 was classified as high and base saturation was 45.75% included in the medium category.

Comparing the results of the analysis on Bayur and Muang Dalam concerning the same disease attack showed that most of the criteria of the soil sampling conditions in both lands had poor soil fertility based on criteria of chemistry and physics of soil by the Institution of Soil Research. Meanwhile, Bogor and were not included in the categories and criteria for growing conditions of maize. Therefore, poor soil fertility affects the health condition of maize plants.

The soil was managed effectively to maintain its fertility by having a good understanding of the environmental condition of the soil in terms of its physical, chemical and biological components (Rosfiansyah and Sopialena, 2018). The more fertile the soil, the healthier the plant, and its resistance to pests and diseases is reduced. Therefore, good soil fertility inhibits the spread of leaf rust disease. Also, in accordance with the criteria for growing conditions, it spurs plants to protect themselves against disease due to adequate nutrition from the soil (Frac et al., 2018).

Similar research on the application of chemical fertilizers significantly affected GLS progress (Graef et al., 2018). Also, they reported that the GLS epidemic was significantly higher in non-fertilized plots than in fertilized plots. They also observed that a single application of nitrogen increased the predisposition of plants toward GLS. However, a combined application of nitrogen and phosphorus at a recommended level significantly reduced the predisposition effect of a high nitrogen level. The unbalanced use of nutrients results in its deficiency in the host and loss of resistance status predisposed the plants to GLS (Graef et al., 2018). Subsequently, Dubey et al. (2019) and Panth et al. (2020) states that soil fertility increases the plant's resistance to pathogens.

**Relationship between temperature, humidity against disease development**

The observations and calculations showed the relationship between temperature and humidity factors on disease development. According to the regression results, the humidity and temperature factors were dominant and the regression equation Y = 0.062x + 25.17 demonstrated a significant link with disease development factors, indicating a relationship between temperature and disease intensity (Figure 4). Meanwhile, the equation Y = 0.809x + 35.09 showed the relationship between humidity and the intensity of the disease (Figure 5). These results were used to predict the development of leaf rust disease in maize plants. However, the two regressions, with an $R^2$ value of 0.76 that shows the relationship between temperature and disease intensity and an $R^2$ of 0.87 that shows the relationship between humidity and disease intensity, were significantly different.

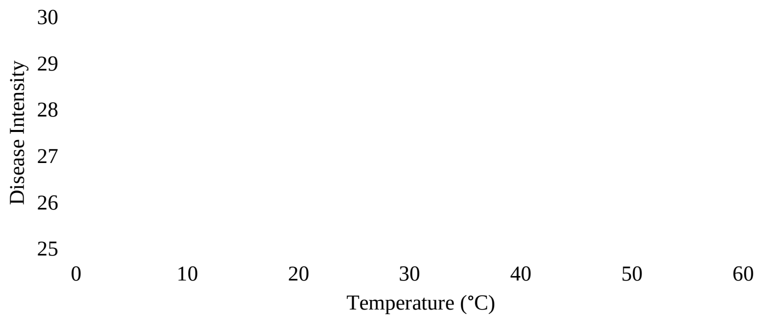



Figure 4. Regression relationship between temperature and intensity of disease

According to Figure 4, the average temperature during the three months observation was 29.120°C, while the emergence of leaf rust symptomatic plants was first characterized by pustules when the average daily temperature reached 27°C with an average daily humidity of 64%, totaling 4 plants from 24 plant samples in Bayur. In Muang Dalam, at first, the emergence of leaf rust symptomatic plants was also characterized by the appearance of pustules occurring when the average daily temperature reached 27°C with the average daily humidity of 78%, totaling 4 plants from 20 plant samples. Then, high average humidity leveled up to > 80%, which contributed to the appearance of leaf rust.

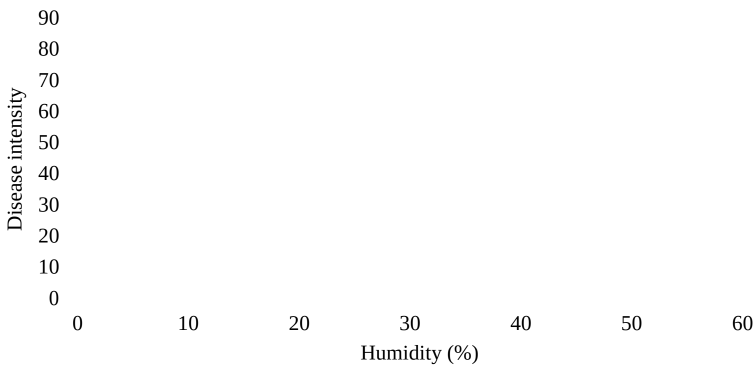



Figure 5. Regression relationship between humidity and intensity of disease

## CONCLUSIONS

It is concluded that the fungus causing leaf rust disease in maize is *P. sorghi* Schw. The average rate of rust infection between Bayur and Muang Dalam Village, Lempake, was not different. Temperature and humidity play a role in the development of leaf rust diseases. However, humidity plays a more important role. The higher the temperature accompanied by the increased humidity, the more leaf rust disease develops. Therefore, good soil fertility in accordance with the criteria for growing conditions spurs plants to protect themselves against disease by adequate soil nutrition.

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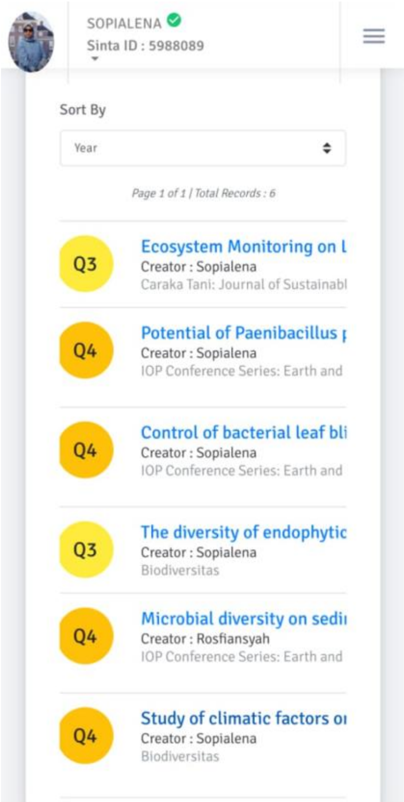
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Dear Mrs sopialena sopialena,

Dear Mrs sopialena sopialena,

Thank you for submitting the manuscript, "ECOSYSTEM MONITORING OF LEAVES OF RUST DISEASE ON CORN CROPS (Zea mays L)" to Caraka Tani: Journal of Sustainable Agriculture. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

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# [CarakaTani] Editor Decision

Inbox

**Mujiyo** 11/6/2019
to me, Suyadi

The following message is being delivered on behalf of Caraka Tani: Journal of Sustainable Agriculture.

________________________________________________________________________

Dear Mrs Sopialena Sopialena,

We have reached a second decision regarding your submission to Caraka Tani:

Journal of Sustainable Agriculture, "ECOSYSTEM MONITORING OF LEAVES OF RUST DISEASE ON CORN CROPS (Zea mays L)".

Our decision is: Resubmit for Review

This article has the potential to be published (and cited). Your article should be corrected according to reviewers' comments. Please see the file attached for the reviewers' comments.

We kindly ask you to resubmit corrected article under the same

Best Quartile Rank in Scopus Inbox

Cara... 6/18/2023

to me

Dear Ir. Hj. Sopialena, MP, Ph.D.,

We are pleased to inform you that your article entitled "Ecosystem Monitoring on Leaves of Leaf Rust Disease of Maize (Zea mays L.)" has been covered in the Scopus database. You can view source details of Caraka Tani: Journal of Sustainable Agriculture in Scopus.

Article website link: https://jurnal.uns.ac.

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friends. Caraka Tani still needs a more solid citation record of its articles in journals/conferences already indexed in Scopus and Web of Science. Most importantly after being covered in Scopus, Caraka Tani needs to put in a lot of effort to obtain the best Quartile rank. It is possible by undertaking measures to improve its citation performance.

We will highly appreciate it if your article has a high citation performance. It will be precious for Caraka Tani: Journal of Sustainable Agriculture as a reputable international journal. Thank you.

# Ecosystem Monitoring on Leaves of Leaf Rust Disease of Maize (*Zea mays* L.)

**Sopialena, Suyadi and Septri Alfian Noor***
Department of Agroecotechnology, Faculty of Agriculture, Universitas Mulawarman, Samarinda, Indonesia

**Corresponding author*: sopialena88@gmail.com

## Abstract

Endemic leaf rust disease always occurs in almost all maize plantations in Indonesia. Furthermore, the development of this disease differs concurrently and is greatly influenced by the ecological conditions of maize cultivation. Therefore, this study fills the epidemiological gap of diseases that has not been conducted against the epidemiology of maize rust. This identifies the causes of leaf rust that attacked the maize plants in two locations, namely Bayur and Muang Dalam, Lempake, Samarinda, Indonesia. This study also analyzed the relationship or model between ecological factors of temperature, humidity, and soil fertility on the intensity of leaf rust and the infection rate of maize leaf rust. Measurement of disease intensity, the rate at which it developed, soil fertility and temperature and humidity of the area are conducted in this study. Meanwhile, soil fertility also influenced disease progression, and the nutrient-poor soils in two sites cause leaf rust disease to develop well. The identification results showed that the cause of maize leaf rust was *Puccinia sorghi* Schw. Therefore, the temperature accompanied by the increased humidity is directly proportional to the development of the leaf rust.

**Keywords:** maize leaf disease identification; plant disease epidemiology; *Puccinia sorghi* Schw; relative humidity; temperature

## INTRODUCTION

Maize leaf rust is an airborne disease because its transmission mode is through the air and grows profusely when the pathogens encounter their host, such as maize. The pathogen penetrates the leaf, infects the plant and multiplies on the leaves (Surtikanti, 2011). The development of this disease is strongly influenced by its ecological conditions, including the temperature and humidity factors, as well as a plant supporting factor, namely soil fertility (Dhami et al., 2015). It is observed that 100% relative humidity and a temperature of 25 to 30°C sporulation of Gray Leaf Spot (GLS) on maize are high, but the number and expansion of lesions are not significantly different at temperature ≥ 25°C. GLS develops slowly when the average daily temperature falls below 20°C. In summer maize, the disease incidence is relatively high in the mountains and the valleys, while it is very low in the terai. However, the disease has attacked the winter and spring maize in the Terai of Nepal (Subedi, 2015).

Agricultural ecosystems are simpler and less stable, making them prone to disease development (Tilman et al., 2002). The stability of an ecosystem is determined by the diversity of structures and the characteristics of its components. Furthermore, this is achieved when an understandable and controllable interaction exists between components (Robertson et al., 2014).

Agroecosystems are dynamic, constantly changing in time and place, and are highly sensitive to influences within and outside the ecosystem. Therefore, to achieve ecosystem management goals, information about its state and dynamics obtained through monitoring activities is required. Furthermore, these activities are conducted to gain information about the state of the ecosystem, including weather, water, soil, pest and disease populations, natural enemies, crop damage and plant growth (Médiène et al., 2011).

According to statistical data, maize production in East Kalimantan increased in 2015, reaching 112,522 tons from the previous year (Badan Pusat Statistik Provinsi Kalimantan Timur, 2016). Meanwhile, this has occurred due to an increase in productivity of 39.35%. Also, according to the field data, one of the constraints of maize production is the attack of leaf rust disease (Kusyanto and Hasmara, 2017).

Leaf rust is a disease that attacks maize plants caused by the fungus *Puccinia sorghi* (Soenartiningsih et al., 2013). The factors that influence leaf rust disease are abiotic and biotic environmental factors such as climate as well as pests, respectively. Asynchronous climate change increases the development rate of fungus, inhibiting the growth of the maize plant itself. Therefore, it is necessary to control the leaf rust disease (Burhanuddin, 2015).

The agroecological approach strives to improve crop yields simultaneously, while also understanding the processes that permit their maintenance (Wezel et al., 2014). However, the primary goal is to determine the long-term sustainability of agricultural systems. The primary foundation of agroecology is the ecosystem concept, defined as a functional system of complementary relationships between living organisms and their environment, delimited by arbitrarily chosen boundaries in space and time and appears to maintain a steady yet dynamic equilibrium (Gliessman, 1995; Ponisio and Ehrlich, 2016). In addition, (Sopialena, 2018) stated that the environment is one of the dominant factors in disease and its progression. Therefore, the research on ecosystems contributes to the development of sustainable agricultural systems (Perfecto and Vandermeer, 2015). The emergence of a disease requires at least three factors, such as the host plant, pathogen and environmental factors (Wakman and Burhanuddin, 2010).

Research on temperature and light on maize conducted by Negeri et al. (2013) showed that the maize phenotype was strongly influenced by temperature. Plants have hypersensitivity symptoms as a result of low temperature. Hence, there is an interaction between temperature and maize genetics. The hypersensitive reaction of maize controlled by the resistance genes is suppressed when maize is grown at a temperature greater > 30°C. Therefore, the phenotype of maize is influenced by an increase or decrease in temperature.

To date, no studies have been conducted on maize's epidemiological leaf rust disease. Therefore, the importance of this study is to fill the gap in knowledge of diseases epidemiology on maize to identify the causes of leaf rust that attack in two research locations, namely Bayur and Muang Dalam, Lempake Samarinda, Indonesia. Also, to examine the relationship or model between ecological factors (temperature, humidity and soil fertility) and the intensity of leaf rust disease as well as its infection rate. Furthermore, the calculation of the ecosystem monitoring model and damage



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level was performed as a measurement reference used as an early warning system in the initial control steps.

## MATERIAL AND METHODS

### Location of the research

This study was conducted on dry land maize plants in Bayur (latitude of -0.4736368 and longitude of 117.1645419) and Muang Dalam Village (latitude of -0.408272 and longitude of 117.1645419), Lempake, Samarinda, East Kalimantan, Indonesia. Also, at Laboratory of Plant Pests and Diseases, Faculty of Agriculture, Universitas Mulawarman. In addition, soil fertility was analyzed at Laboratory of Soil Science, Faculty of Agriculture, Universitas Mulawarman.

### Research design

***Survey***

A survey was conducted from January to April 2019 to determine the sampling sites. The first location was a maize field in Bayur Village, Lempake, which had previously been planted with long beans and had 144 maize plants (Bonanza F1 types). Meanwhile, the fertilizers and pesticides used for maize crops are NPK as well as Basmilang, respectively. The second sampling location was a maize field in Muang Dalam, Lempake, with a population of 100 maize plants (Bonanza F1 varieties) and had previously been planted with paddy. The fertilizers used included NPK, and the pesticides are Toxafine and Gramoxone. The sites' conditions are shown in Figure 1, and the distance between Bayur and Muang Dalam Village is 6 km.

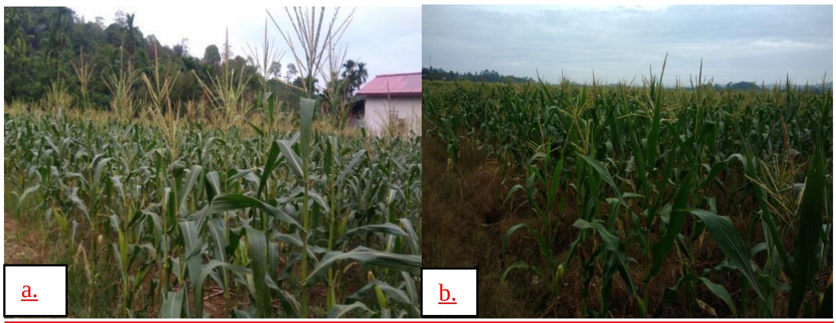


Figure 1. Maize plantation in Bayur (a) and Muang Dalam (b)

This disease was identified in the laboratory, where pustules from infected leaves were scrapped using a needle sprayed with alcohol. After which, there were placed on an object glass and given a drop of methylene blue and safranin. The morphology of the pathogen was observed using an optical microscope.

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***Observation parameters***

Using a wet-dry ball thermometer, the temperature and humidity were measured daily at the two locations starting from 1st week after planting. The data were collected three times a day, including morning 6.00 AM, afternoon 12 AM and evening 6 PM. Furthermore, the pathogen was identified in the Laboratory of Plant Protection, Faculty of Agriculture, Universitas Mulawarman by scraping the pustule on the surface of the maize leaf using an ooze needle. Afterward, there were placed on a glass object and observed under the microscope. The soil nutrient and pH analysis were conducted in the Laboratory of Soil Science, Faculty of Agriculture, Universitas Mulawarman. The method used is total N (Khejdhal Method), potential-P (Extraction of HCl 25% using Bray or Olsen methods), available-K (1 N $NH_4OAc$ pH7) and soil pH (Electrometric Method using pH meter Hanna type HI 8424, by comparison, the liquid 1 : 2.5).

## Data analysis

***Disease intensity***

The following formula is used to calculate the disease intensity:

$$I = \frac{\sum (ni \text{ x } vi)}{Z \text{ x } N} \text{ x } 100\% \qquad (1)$$

Where I represent the disease intensity (%), ni represent the numbers of plant leaves attacked, vi represent the scale of the attack, N represent the total number of leaves observed and Z represent the highest scale of attack categories

Table 1. Scale category of leaf rust disease (Jeffers and Plank,1965)

| Scale | Attack of the leaf area |
|---|---|
| 1 | 1 – 5% |
| 3 | 5 – 11% |
| 5 | > 11 – ≤ 25% |
| 7 | > 25 – ≤ 75% |
| 9 | > 75 – 100% |

***Infection rate***

According to Jeffers and Plank (1965), the following formula is used to calculate the disease infection rate:

$$r = \frac{2.3}{t_2 - t_1}\left(\log_{10}\frac{x_2}{1 - x_2} - \log_{10}\frac{x_1}{1 - x_1}\right) \qquad (2)$$

Where r represent the infection rate, 2.3 represent the number of natural logarithmic conversion results to ordinary logarithm (Lnx = 2.3 Logx), t represent observation time interval, $x_2$ represent the proportion of diseased leaves at t, $x_2$ represent the proportion of diseased leaves at baseline.

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Table 2. Infection rate criteria adopted from (Jeffers and Plank, 1965)

| Infection rate (unit of the week) | Criteria |
|---|---|
| < 0.11 | Mild |
| >0.11 – < 0.50 | Moderate |
| >0.50 | Severe |

## RESULTS AND DISCUSSION

### Fungal identification

*Puccinia sorghi* Scwh often appeared after silking in maize, having an early symptom of chlorotic specks on the leaf. Meanwhile, the obvious sign of this pathogen was golden-brown pustules or bumps on the above-ground surface of the plant tissue. These bumps were uredospores that could spread to other plants and cause further infection. Also, they were circular and powdery due to spores breaking through the leaf surface. The bumps are only about 1 to 2 mm each and are numerous with equal frequencies on upper and lower leaf surfaces (Burhanuddin, 2015; Utpal et al., 2015).

Figure 2 shows details of *Puccinia sorghi* Scwh based on the laboratory analysis of maize rust on leaves from two areas, Bayur and Muang Dalam plantations, preferring humidity > 75% and temperature > 20$^{o}$C. The spores were unable to live without their host, hence, these are termed obligate parasites. First, *Puccinia sorghi* scrubbed the pustules on the surface of maize leaves with a needle placed them on the object-glass, after which methylene blue and safranin staining was added and lastly viewed directly under a microscope.

Observations under a microscope with a 400x enlargement showed oval and round shapes. This is in accordance with Jeffers and Plank (1965), stating that the *Puccinia sorghi* Scwh uredospore was yellowish to golden colour. The walls of the spores thickened it at both ends to golden. Also, its walls are thick with 4 to 5 holes-equator. Teliospore is brown, smooth, elliptical and round at both ends.

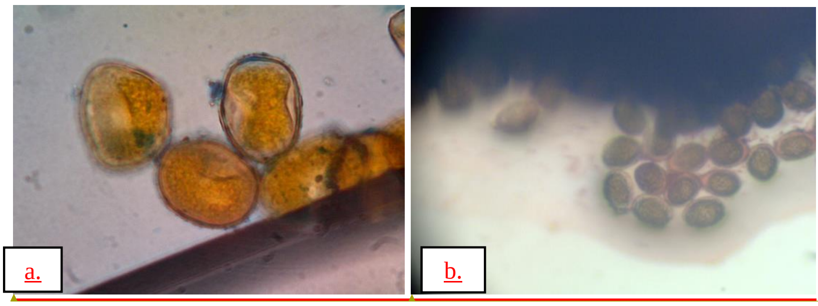


Figure 2. Microscopic *Puccinia sorghi* Schw methylene blue staining (a), safranin staining (400x) (b)

Fungal spores that spread through the air landed on the surface of healthy leaves. Although they were observed 11 times, leaf rust occurred at 55 days or 9th week after inoculation, as shown in the Table 3.

Table 3. The observation results on leaf rust distribution in Bayur and Muang Dalam fields.

| Observation week to | Number of pustules | |
|---|---|---|
| | Bayur | Muang Dalam |
| 1 | - | - |
| 2 | - | - |
| 3 | - | - |
| 4 | - | - |
| 5 | - | - |
| 6 | - | - |
| 7 | - | - |
| 8 | - | - |
| 9 | 149 | 276 |
| 10 | 2,413 | 3,655 |
| 11 | 11,151 | 9,361 |

The early pustules grew gradually and only existed in the margins of maize leaves, as shown in Figure 3. Pustules were circular to round and orange to brown, and they rarely grow under the surface of maize leaves. Spores spread throughout the surface of maize leaves during their harvested period. RPK (1972) and Utpal et al. (2015) stated that the leaf rust disease on maize caused by the fungus *Puccinia sorghi* Schw occurred every growing season. Rust pustules usually occur in high relative humidity and high-temperature conditions, with characteristic chlorotic specks on the leaf surface. These soon developed into powdery, brick-red pustules as the spores broke through the leaf surface. The pustules were oval or elongated, about 1/8 inch long and scattered sparsely or clustered together. The leaf tissue around it was likely to be yellow or die, leaving lesions of dead tissue forming a band across the leaf, resulting in their death when severely infected. According to the age of the pustules, the red spores turned black and continued to erupt through the leaf surface. Some parts which are also prone to infection are the husks, leaf sheaths and stalks (Adegbite, 2011; Suriani et al., 2019).

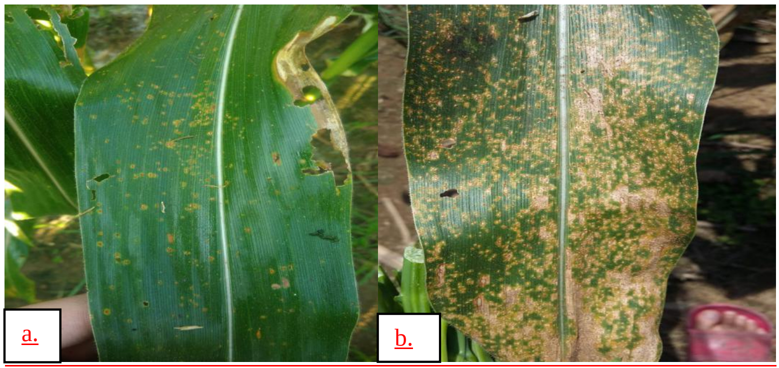


Figure 3. Early pustules appear (a) and pustules spread throughout the leaf surface (b)

### Disease intensity

Disease occurrence is the proportion of individual hosts or organs affected by the disease



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regardless of how severe it might be, while its severity is the proportion of infected host surfaces to the total observed host surface, having symptoms expressed as a wide percentage to leaf surface area. Furthermore, this also refers to disease intensity (Agrios, 2005).

It has been observed that the average disease intensity ranged from 27 to 81% (Table 3), from moderate to severe criteria. The disease attacked the lower leaves until they went up, leaving the younger ones on the top. This was due to the disease's preference for humid and shady environments. Plant diseases were scored (assessed) by comparing plant symptoms to score tables from the Directorate of Protection. (Table 1).

**The infection rate of leaf rust disease**

The infection rate was obtained based on the proportion of diseased plants (percentage of disease), calculated every week according to the progression. The calculation of disease infection rates in the two research sites is shown in Table 4. Since the average rate of rust infection between the two places was not different, it implies that rust has been endemic in the area.

Table 4. Results for calculation of Bayur infection rates and Muang Dalam infection rate

| Place | Infection rate (unit week) | | Mean average | Mean average temperature (°C) | Mean average RH (%) |
|---|---|---|---|---|---|
| | r1 | r2 | | | |
| Bayur | 2,887 | 1,953 | 0,739 | 29.12 | 70 |
| Muang Dalam | 2.16 | 1,424 | 1,792 | 29.39 | 70 |
| Mean average | 2,523 | 1.688 | 1,081 | | |

Note: r1 = infection rate 1; r2 = infection rate 2; RH = relatif humidity

The observations are taken from 24 and 20 samples of plant leave in Bayur and Muang Dalam fields, respectively, which appeared to be increasing due to environmental conditions, especially temperature and humidity. The disease's development is also aided by significant rainfall, which causes infection to increase over time (Kinyua et al., 2011; Sopialena and Palupi, 2017).

The average infection rate in both places was more than 0.50 units per week$^{-1}$ due to the rapid spread of spores. The attacks by the fungus of *Puccinia sorghi* were too aggressive. Host varieties that are not resistant to disease and environmental factors support the development of pathogens. According to Jeffers and Plank (1965), the infection rate is used to identify the vulnerability of aggressive organisms, as well as the conducive status of the environment to disease development. A value of r greater than 0.5 units day$^{-1}$ (Table 2) implies that an aggressive pathogen and the host variety are susceptible to disease as well as weather support and vice versa.

The infection rate in the Bayur field at the beginning and end of the observation was 2.887 units week$^{-1}$ and 1.953 units week$^{-1}$, respectively. Meanwhile, the level of infection in Muang Dalam at the beginning and end of the observation was 2.16 units week$^{-1}$ and 1.424 units week$^{-1}$ 4, respectively.

The results above are similar to the research conducted by Pap et al. (2013); Thorson and Martinson (1993), which revealed that relative humidity of 95% was optimal for germ tube elongation

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**Observation of thse spread area**

Based on the study results, pustules in leaf rust appeared in the 9th week, increasing weekly. Firstly, the pustule was visible on the edge of the leaf, then it spread to the center of the leaf. Finally, in the 13th week, its number increased to 80% of the leaves' surface covered by the rust pustule.

The symptom of *Puccinia sorghi* Shcw pathogen attack was the absence of a golden brown pustule on the leaf's surface. Furthermore, this lump was a collection of uredospore that penetrated the leaf's surface, turning the yellow patches next to the spots to brownish. Meanwhile, the brown lump could turn blackish due to many pustules on the leaf. In stricken crops, symptoms increases covering the entire maize leaf (Menkir et al., 2006; Puspawati and Sudarma, 2016).

**Soil analysis results**

The results of soil analysis of total-N (Khejdhal Method), potential-P (Extraction of HCl 25%), available K (1 N $NH_4OAc$ pH7) and soil pH (Electrometric Method) is shown in Table 5.

Table 5. Soil analysis of Bayur and Muang Dalam

| Location | pH | Total-N (%) | Potential-P (ppm) | Available K (ppm) |
|---|---|---|---|---|
| Bayur | 5.01 | 0.44 | 40.37 | 171.5 |
| Muang Dalam | 5.44 | 0.33 | 35.19 | 261.09 |

| Location | Base cation (pH 7) | | | | CEC | Base saturation (%) |
|---|---|---|---|---|---|---|
| | $Ca^{++}$ | $Mg^{++}$ | $K^{+}$ | $Na^{+}$ | | |
| | me $100g^{-1}$ | | | | | |
| Bayur | 5.4666 | 4.12 | 0.39 | 0.3411 | 15.06 | 66.17 |
| Muang Dalam | 6.87 | 3.88 | 0.85 | 0.4731 | 26.40 | 45.75 |

Table 5 shows that Bayur with pH 5.01 was included in the acid category. N total of 0.44%, P and K was 40.37 ppm included in the high category and 171.50 ppm included in the very high category. $Ca^{++}$ 5.4666 ppm was classified as moderate, $Mg^{++}$ 4.12 ppm was classified as high, $K^{+}$ 0.39 ppm was categorized as low, $Na^{+}$ 0.3411 was classified as low, CEC 15.60 was classified as moderate and Base saturation was 66.17% included in the high category.

In Muang Dalam, pH 5.44 was included in the acid category. N total of 0.33%, P and K was l35.19 ppm included in the high category and 261.09 ppm included in the very high category. $Ca^{++}$ 6.87 ppm was classified as moderate, $Mg^{++}$ 3.88 ppm was classified as high, $K^{+}$ 0.85 ppm was

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categorized as low, $Na^{+}$ 0.4731 was classified as moderate, CEC 26.40 was classified as high and Base saturation was 45.75% included in the medium category.

Comparing the results of the analysis on Bayur and Muang Dalam concerning the same disease attack showed that most of the criteria of the soil sampling conditions in both lands had poor soil fertility based on criteria of chemistry and physics of soil by the Institution of Soil Research. Meanwhile, Bogor and were not included in the categories and criteria for growing conditions of maize. Therefore, poor soil fertility affects the health condition of maize plants.

The soil was managed effectively to maintain its fertility by having a good understanding of the environmental condition of the soil in terms of its physical, chemical and biological components (Rosfiansyah and Sopialena, 2018). The more fertile the soil, the healthier the plant, and its resistance to pests and diseases is reduced. Therefore, good soil fertility inhibits the spread of leaf rust disease. Also, in accordance with the criteria for growing conditions, it spurs plants to protect themselves against disease due to adequate nutrition from the soil (Frac et al., 2018).

Similar research on the Application of chemical fertilizers significantly affected GLS progress (Graef et al., 2018). Also, they reported that the GLS epidemic was significantly higher in non-fertilized plots than in fertilized plots. They also observed that a single application of nitrogen increased the predisposition of plants toward GLS. However, a combined application of nitrogen and phosphorus at a recommended level significantly reduced the predisposition effect of a high nitrogen level. The unbalanced use of nutrients results in its deficiency in the host and loss of resistance status predisposed the plants to GLS (Graef et al., 2018). Subsequently, Dubey et al. (2019) and Panth et al. (2020) states that soil fertility increases the plant's resistance to pathogens.

**Relationship between temperature, humidity against disease development**

The observations and calculations showed the relationship between temperature and humidity factors on disease development. According to the regression results, the humidity and temperature factors were dominant and the regression equation $Y = 0.062X + 25{,}17$ demonstrated a significant link with disease development factors, indicating a relationship between temperature and disease intensity (Figure 4). Meanwhile, the equation $Y = 0.809X + 35{,}09$ showed the relationship between humidity and the intensity of the disease (Figure 5). These results were used to predict the development of leaf rust disease in maize plants. However, the two regressions, with an R2 value of 0.76 that shows the relationship between temperature and disease intensity and an R2 of 0.87 that shows the relationship between humidity and disease intensity, were significantly different.

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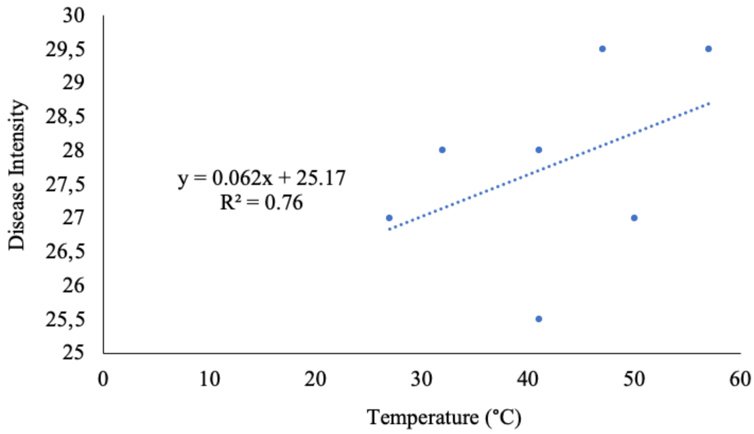



Figure 4. Regression relationship between temperature and intensity of disease

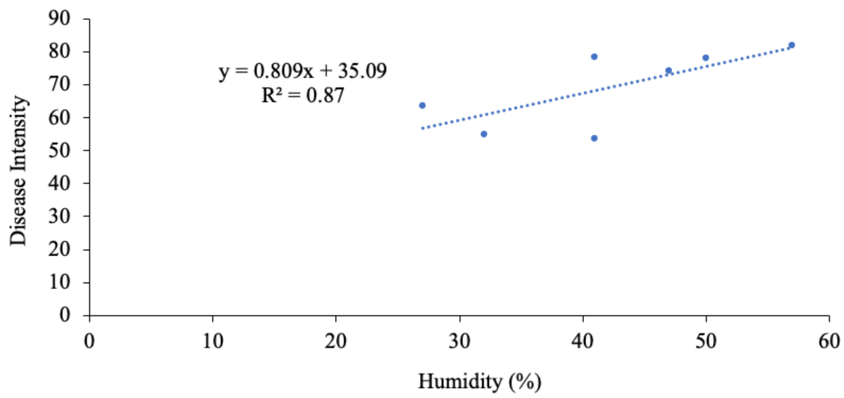
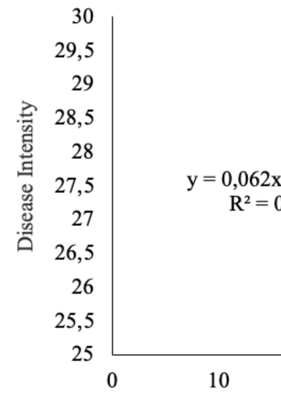



Figure 5. Regression relationship between humidity and intensity of disease

According to Figure 4, the average temperature during the three months observation was 29,120°C, while the emergence of leaf rust symptomatic plants was first characterized by pustules when the average daily temperature reached 270°C with an average daily humidity of 640°C, totaling 4 plants from 24 plant samples in Bayur. In Muang Dalam, at first, the emergence of leaf rust symptomatic plants was also characterized by the appearance of pustules occurring when the average daily temperature reached 27°C with the average daily humidity of 78°C, totaling 4 plants from 20 plant samples. Then, high average humidity leveled up to > 80%, which contributed to the appearance of leaf rust.

## CONCLUSIONS

It is concluded that the fungus causing leaf rust disease in maize is *Puccinia sorghi* Schw. The average rate of rust infection between Bayur and Muang Dalam Village, Lempake, was not different.

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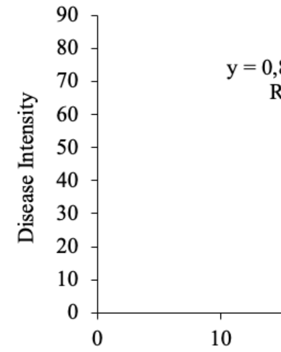


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Temperature and humidity play a role in the development of leaf rust diseases. However, humidity plays a more important role. The higher the temperature accompanied by the increased humidity, the more leaf rust disease develops. Therefore, good soil fertility in accordance with the criteria for growing conditions spurs plants to protect themselves against disease by adequate soil nutrition.

## ACKNOWLEDGEMENTS

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## COVERING LETTER

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# Ecosystem Monitoring on Leaves of Leaf Rust Disease of Maize (*Zea mays* L.)


## Abstract

Leaf rust disease of corns is an endemic disease that always occurs in almost all corn plantations in Indonesia. The development of corn rust disease differs from place to place, and is greatly influenced by the ecological conditions of corn cultivation. This research is important to do to fill the epidemiological gap of diseases that has not been done against the epidemiology of rust disease of corn. The purposes of this research were to identify the causes of leaf rust that attacked the corn plants in two research locations namely Bayur and Muang Dalam, Lempake, Samarinda, Indonesia and to see the relationship or model between ecological factors (temperature, humidity and soil fertility) on the intensity of leaf rust disease and the infection rate of corn leaf rust. The research was conducted by measuring disease intensity, speed of disease development, soil fertility, and temperature and humidity of the research area.Temperature and humidity play roles in the development of leaf rust diseases, but humidity plays a more important role in the development of leaf rust diseases of corn. The soil fertility also influenced disease progression. The nutrient-poor soils in two research sites cause leaf rust disease to develop well. The identification results revealed that the cause of corn leaf rust was *Puccinia sorghi* Schw. The higher the temperature accompanied by the increased the humidity, the more leaf rust disease develops.



Keywords: corn leaf disease identification; plant disease epidemiology; *Puccinia sorghi* Schw; temperature; relative humidity


## INTRODUCTION

Corn leaf rust disease is called airborne disease because its mode of transmission is through air and will greatly develop if the pathogens that cause disease meet its host such as corn. The pathogen will enter the leaf and infect the plant and will multiply on the leaves [1]. The development of this disease is greatly influenced by its ecological conditions, especially the temperature and humidity factors as well as a plant supporting factor, namely soil fertility [2]. It is observed that 100% relative humidity and 25°C-30°C temperature sporulation of Gray Leaf Spot (GLS) on corn are high but the number of lesions and lesion expansion are not significantly different with >25°C temperature. GLS is slow to develop when the mean of daily temperature drops below 20°C. In summer corn the incident of the disease is quite high in the mountains and the valleys with very little disease incidence noted in the terai; however the disease has attacked the winter and spring maize of the terai in Nepal [3].

Agricultural ecosystems are simpler and less stable ecosystems compared to natural ecosystems. Therefore, the agricultural ecosystem is prone to disease development (Tilman et al., 2002). The stability of an ecosystem is not only determined by the diversity of structures but also by the characteristics of the ecosystem components. If the interaction between components can be understood and managed appropriately, ecosystem stability can be sought [5].

Agroecosystems are dynamic, always changing between time and places, and very sensitive to influence from inside and outside the ecosystem. In order to achieve ecosystem management objectives, information about the state and dynamics of ecosystems obtained from monitoring activities is needed. Monitoring activities are carried out to gain information about the state of the

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ecosystem including weather, water, soil, pest and disease populations, natural enemies, crop damage and plant growth (Mediene et al., 2011).

Based on the statistical data, in 2015, corn production in East Kalimantan increased, amounted to 112.522 tons from the previous year (Badan Pusat Statistik Provinsi Kalimantan Timur, 2016). The increase in production is expected to occur due to an increase in productivity of 39.35%. Despite the increased productivity, according to the field data, one of the constraints of corn production is the attack of leaf rust disease (Kusyanto et al., 2017).

Leaf rust is a disease that attacks corn plants caused by the fungus *Puccinia sorghi* (Soenartiningsih et al., 2013). The factors that influence the occurrence of leaf rust disease is abiotic environmental factors such as climate and biotic environment such as pest. Asynchronous climate change makes the rate of fungus growth grows faster which results in inhibiting the growth of the corn plant itself. Therefore it is necessary to control the leaf rust disease [10].

The agroecological approach strives to improve crop yields simultaneously, as well as to understand the processes which permit the maintenance of those yields [11]. The primary goal is establishing a mean of determining the long-term sustainability of agricultural systems. The primary foundation of agro ecology is the ecosystem concept, defined as a functional system of complementary relations between living organisms and their environment, delimited by arbitrarily chosen boundaries in space and time and appears to maintain a steady yet dynamic equilibrium [12]. In addition, Sopialena (2018) pointed out that the environment is one of the dominant factors in the process of disease and disease progression. The ecosystem research will contribute to the development of sustainable agricultural systems (Perfecto et al., 2015). The emergence of a disease requires at least three factors: the host plant, the pathogen and the environmental factors (Wakman et al., 2010).

The studies of epidemiological leaf rust disease on corn have not been carried out yet. This study is to fill the gap in knowledge of diseases epidemiology on corn. The purposes of this study were to identify the causes of leaf rust that attacked the corn plants in two research locations namely Bayur and Muang Dalam, Lempake, Samarinda, Indonesia and to see the relationship or model between ecological factors (temperature, humidity and soil fertility) on the intensity of leaf rust disease and the infection rate of corn leaf rust. Model of ecosystem monitoring and damage level calculation are carried out as a reference for measurement and can be used as an Early Warning System in the initial steps of control.

## MATERIAL AND METHODS

### Location of the research

This study was conducted on dry land corn plants in Bayur and Muang Dalam Village, Lempake District, Samarinda, East Kalimantan, Indonesia and at Laboratory of Plant Pests and Diseases, Faculty of Agriculture, Mulawarman University. Soil fertility was analyzed at Soil Science

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Laboratory, Agriculture Faculty, Mulawarman University.

**Research design**

**Survey**

Survey was conducted on January to April 2019/ This survey to determine sampling sites. The first sampling location was corn field in Bayur Village, Lempake. The plant population was 144 corn plants (Bonanza F1 varieties). This field was previously planted with long bean. Fertilizers used for corn crops were NPK, and pesticides called Basmilang. The second sampling location was corn field in Muang Dalam, Lempake Village. The plant population was 100 corn plants (Bonanza F1 varieties). This land used to be paddy fields and is now used for corn plantation. Fertilizers used for corn crops included NPK, and pesticides, namely Toxafine and Gramoxone. The sites condition are shown in Figure 1. The distance between Bayur and Muang Dalam Village is 6 km.

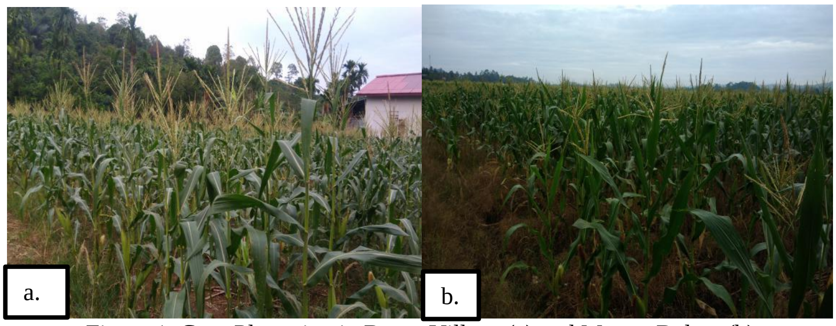


Figure 1. Corn Plantation in Bayur Village (a) and Muang Dalam (b)

Identification of leaf rust disease was done in the laboratory. Pustules from infected leaf were scrapped using a needle that has been sprayed with alcohol. Next, scraped pustules were placed on an object glass and given a drop of methylene blue and safranin. The morphology of the pathogen was seen using Optilab microscope.

**Observation parameters**

Temperature and humidity were observed at the two locations of research. observation of temperature and humidity was carried out every day starting from 1 week after planting by using a wet-dry ball thermometer. The data were taken 3 times a day: morning 6.00 AM, afternoon 12 AM and evening 6 PM. Identification of pathogen was carried out in the laboratory of Plant Protection, Agriculture Faculty, Mulawarman University by scraping the pustule on the surface of the corn leaf using an ooze needle, then the pustules were placed on object glass to be observed under the microscope. Nutrient and soil pH analysis were carried out in the laboratory of Soil Science, Agriculture Faculty, Mulawarman University.

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**Data analysis**

Disease intensity

The disease intensity is calculated using the formula:

$$I = \frac{\sum(ni \times vi)}{Z \times N} \times 100\%$$

where:

I = disease intensity (%)

ni = Numbers of plant leaves attacked

vi = The scale of the attack

N = The total number of leaves observed

Z = The highest scale of attack scale categories

Table 1. Scale category of leaf rust disease

| Scale | Attack of the leaf area |
|---|---|
| 1 | 1 – 5% |
| 3 | 5 – 11% |
| 5 | >11 – ≤ 25% |
| 7 | >25 – ≤ 75% |
| 9 | >75 – 100% |

Source : [16]

Infection rate

Calculation of disease progression rates according to [16] can be calculated using the formula:

$$r = \frac{2.3}{t2 - t1}\left(\log \frac{x_2}{1 - x_2} - \log \frac{x_1}{x_1}\right)$$

where:

r = Infection rate

2.3 = The number of natural logarithmic conversion results to ordinary logarithm (Lnx = 2.3 Logx)

t = Time Observation interval

xt = Proportion of diseased leaves when t

x0 = Proportion of diseased leaves at baseline

Table. 2 Infection rate criteria adopted from [16]

| No. | Infection rate (unit of the week) | Criteria |
|---|---|---|
| 1. | < 0.11 | mild |
| 2. | >0.11 - < 0.50 | moderate |
| 3. | >0.50 | severe |

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## RESULTS AND DISCUSSION

### Fungal identification

*P. sorghi* Scwh often first appeared after silking in corn. The first early symptom included chlorotic specks on the leaf. The obvious sign of this plant pathogen was golden-brown pustules or bumps on the above-ground surface of the plant tissue. These bumps were uredospores which could spread to other plants and caused further infection. They were circular and powdery, which result from spores breaking through the leaf surface. While they are only about 1 - 2 mm each, they are very numerous with equal frequencies on upper and lower leaf surfaces [10].

Based on analysis conducted in laboratory, by identifying the corn rust on leaves from two areas, Bayur and Muang Dalam Plantations, the fungus *P. sorghi* Scwh details could be seen in Figure 2 *P. sorghi* Scwh liked humidity above 75% and temperature > 20$^{o}$C. Spores were unable to live without their host. If the carrying host dies, these mold spores also die. In other words, this is called as obligate parasites. *P. sorghi* scrubbed the pustules on the surface of corn leaves with a needle and it was then placed on the object glass, added by methylene blue and safranin staining and lastly viewed directly under a microscope.

Observations under a microscope with an enlargement of 400x showed oval and round shapes. This is in accordance with (Barnett and Hunter, 1972) that the *P. sorghi* Scwh uredospore was yellowish to golden colour. The walls of the spores thickened both ends of the spores to golden. They had thick spore walls with 4- 5 holes - equator. Teliospore is brown, smooth, elliptical and round at both ends.

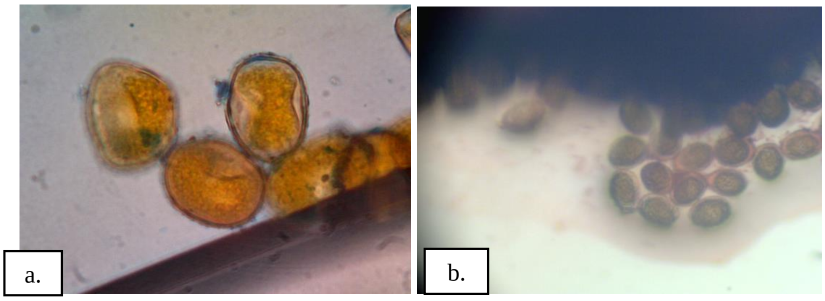


Figure 2. Microscopic *P. sorghi* Schw methylene blue staining (a); safranin staining (400x) (b)

Fungal spores that spread through the air landed on the surface of healthy leaves. In this study, they were observed 11 times, but leaf rust occurred at the age of 55 days after inoculation or at week 9$^{th}$ which can be seen in the following table.

Table 3. Results of observations on the area of leaf rust distribution in Bayur and Muang Dalam fields.

| Observation week to | Number of Pustules |
|---|---|

| | Bayur Village | Muang Dalam |
|---|---|---|
| 1 | - | - |
| 2 | - | - |
| 3 | - | - |
| 4 | - | - |
| 5 | - | - |
| 6 | - | - |
| 7 | - | - |
| 8 | - | - |
| 9 | 149 | 276 |
| 10 | 2,413 | 3,655 |
| 11 | 11,151 | 9,361 |

Early pustules grew small and only existed in the margins of corn leaves as can be seen in Figure 2. Pustules were circular to round and orange to brown. It was rarely found that pustules grew under the surface of corn leaves. Spores spread throughout the surface of corn leaves when the age of corn plants entered the harvested period. Agrios (2005) and Oliveira et al. (2020) mentioned that leaf rust disease on corn was caused by the fungus *P. sorghi* Schw and occurred every growing season. Rust pustules usually first appeared in high relative humidity and high temperature. Early symptoms of common rust were chlorotic flecks on the leaf surface. These soon developed into powdery, brick-red pustules as the spores broke through the leaf surface. Pustules were oval or elongated, about 1/8 inch long, and scattered sparsely or clustered together. The leaf tissue around the pustules was likely to be yellow or die, leaving lesions of dead tissue. The lesions sometimes formed a band across the leaf and entire leaves would die if severely infected. According to age of the pustules, the red spores turned black, so the pustules appeared black, and continued to erupt through the leaf surface. Husks, leaf sheaths, and stalks were likely to be also infected.

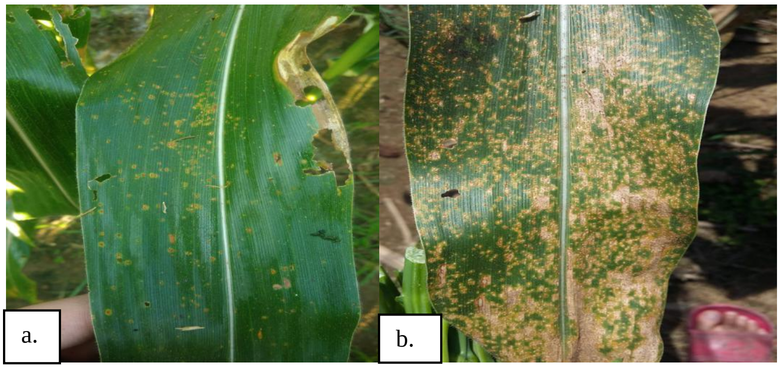


Figure 3. Early pustules appear (a) and Pustules spread throughout the leaf surface (b)

## Disease intensity

Disease occurrence is the proportion of individual hosts or organs affected by the disease regardless of how severe the disease is while the severity of the disease or the severity of the disease

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is the proportion of infected host surfaces to the total observed host surface. The severity of disease symptoms is expressed as a wide percentage of symptoms to leaf surface area. Disease severity also refers to Disease Intensity [18].

Based on observations, it was found that the average disease intensity ranged from 27% to 81% (Figure 2 and 3), from moderate to very severe criteria. The disease attacked the lower leaves until the leaves went up, so that young leaves were left on the top. This result was related to the humidity, where the disease preferred humid and shaded conditions. Scoring (assessment) of plant diseases was conducted by comparing the symptoms occurred in plants and score tables from the directorate of plant protection (Table 1).

**Leaf rust disease infection rate**

Table 4. Results for Calculation of Bayur Infection Rates and Muang Dalam Infection Rate

| Place | Infection Rate (Unit Week) | | Mean Average | Mean Average Temperature ($^o$C) | Mean Average RH (%) |
|---|---|---|---|---|---|
| | r1 | r2 | | | |
| Bayur | 2.887 | 1.953 | 0,739 | 29.12 | 70 |
| Muang Dalam | 2.16 | 1.424 | 1.792 | 29.39 | 70 |
| Mean Average | 2.523 | 1.688 | 1.081 | | |

Note: r1 = Infection rate 1; r2 = Infection rate 2; RH = Relatif humidity

Infection rate was obtained on the basis of the proportion of diseased plants (percentage of disease), which was calculated every week according to disease progression. The results of the calculation of disease infection rates in the two research sites can be seen in Table 5. It was indicated that the average rate of rust infection between the two places was not different. This means that rust has been endemic in the area.

The results of observations taken from 24 samples of plant leaves in Bayur field and 20 samples of plant leaves in Muang Dalam field appeared to be increasing. This is due to environmental conditions at the time of observation, especially temperature and humidity. The process of infection with high rainfall also supports the development of the disease so that infection increases from time to time [20].

The average rate of infection in both places was more than 0.50 units week$^{-1}$. This happened due to the rapid spread of spores. *P. sorghi* fungus attacks were too aggressive. Host varieties that are not resistant to disease and environmental factors support the development of pathogens. According to (Jeffers and Plank, 1965) the value of infection rate can be interpreted whether aggressive pathogens are susceptible or resistant and whether the environment is supportive or not for disease development. If the value of r is greater than 0.5 units per day (Table 2), it means that an aggressive pathogen and the host variety are susceptible to disease and weather support and vice versa.

The attack rate in Bayur field at the beginning of the observation was with an infection rate of 2.887 units week$^{-1}$ while at the end of the observation it was 1.953 units week$^{-1}$. The level of attack in

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Muang Dalam Village at the beginning of the observation was with an infection rate of 2.16 units week$^{-1}$ while at the end of the observation it was 1.424 units week$^{-1}$ 4.

The results above similar to the research that were conducted by [21] which revealed that relative humidity of 95% was optimal for germ tube elongation and formation of appressoria. [22] observed that sporulation was high at 100% relative humidity and 25°C-30°C temperature but the number of lesions and expansion of lesion were not significantly different with >25°C temperature. Disease intensity occurred more on warm and humid conditions [23]. Rust disease was slow to develop when the mean daily temperature dropped below 20°C [24].

**Observation of spreaded area**

Based on the results of the study, pustule in leaf rust appeared in the 9$^{th}$ week and the number of pustule increased every week. At first the pustule was visible on the edge of the leaf, then the pustule spread to the center of the leaf. In the 13$^{th}$ week, the number of pustule increased to 80% of the surface of the leaves covered by the rust pustule of the leaves.

The symptom of *P. sorghi* Shcw pathogen attack was the absence of a golden brown pustule or lump on the surface of the leaf. This lump or pustule was a collection of uredospore and penetrated the surface of the leaf so that the leaves of yellow patches next to the spots turned brownish. The amount of pustule on this leaf was very large. This brown lump could turn blackish. In stricken crops, symptoms will increase thus covering the entire corn leaf [25].

**Soil analysis results**

From the results of soil analysis in the Soil Science Laboratory of the Faculty of Agriculture showed in Table 5 below:

Table 5. Soil Analysis of Lahan Bayur Village and Muang Dalam Village

| Location | pH | N Total (%) | Available P (ppm) | Available K (ppm) |
|---|---|---|---|---|
| Bayur | 5,01 | 0,44 | 40,37 | 171,5 |
| Muang Dalam | 5,44 | 0,33 | 35,19 | 261,09 |

| Location | Base Cation (pH 7) | | | | KTK | Base Saturation (%) |
|---|---|---|---|---|---|---|
| | Ca++ | Mg++ | K+ | Na+ | | |
| | me/100g | | | | | |
| Bayur | 5,4666 | 4,12 | 0,39 | 0,3411 | 15,06 | 66,17 |
| Muang Dalam | 6,87 | 3,88 | 0,85 | 0,4731 | 26,4 | 45,75 |



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From the table above, it was shown that Bayur land (T1) with pH 5.01 was included in the acid category. N total of 0.44% P and K was 40.37 included in the high category and 171.50 included in the very high category. $Ca^{++}$ 5.4666 was classified as moderate. $Mg^{++}$ 4.12 was classified as high. $K^{+}$ 0.39 was categorized as low. $Na^{+}$ 0.3411 was classified as low. CEC 15.60 was classified as moderate. Base saturation was 66.17% included in high category.

In Muang Dalam land (T2) pH 5.44 was included in the acid category. N total of 0.33% P and K was l35.19 included in the high category and 261.09 included in the very high category. $Ca^{++}$ 6.87 was classified as moderate. $Mg^{++}$ 3.88 was classified as high. $K^{+}$ 0.85 was categorized as low. $Na^{+}$ 0.4731 was classified as moderate. CEC 26.40 was classified as high. Base saturation was 45.75% included in the medium category.

Comparison of the results of the analysis on Bayur and Muang lands with regard to the same disease attack revealed that the soil sampling conditions in both lands had poor soil fertility and were not included in the categories and criteria for growing conditions of maize. Poor soil fertility would affect the health condition of corn plants.

By understanding the soil environmental conditions well from the physical, chemical and biological aspects, the management of the land to maintain the fertility of the land can be done appropriately [26]. The more fertile a soil will make a healthy plant and affect the resistance against pests and diseases. Good soil fertility can inhibit the spread of disease attacks including leaf rust disease. Good soil fertility in accordance with the criteria for growing conditions can spur plants to protect themselves against disease due to adequate nutrition from the soil [27].

Similar research on Application of chemical fertilizers significantly affected Grey Leaf Spots (GLS) progress [28] They reported that GLS epidemic was significantly higher in non-fertilized plots than fertilized plots. They also observed that a single application of nitrogen increased the predisposition of plants toward GLS but a combined application of nitrogen and phosphorus at a recommended level significantly reduced the predisposition effect of a high nitrogen level. The unbalanced use of nutrients caused host nutrient deficiency and lose of resistance status predisposed the plants to GLS [28]. Added by [29], soil fertility will increase the plant's resistance to pathogens.

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**Relationship between temperature, humidity against disease development**

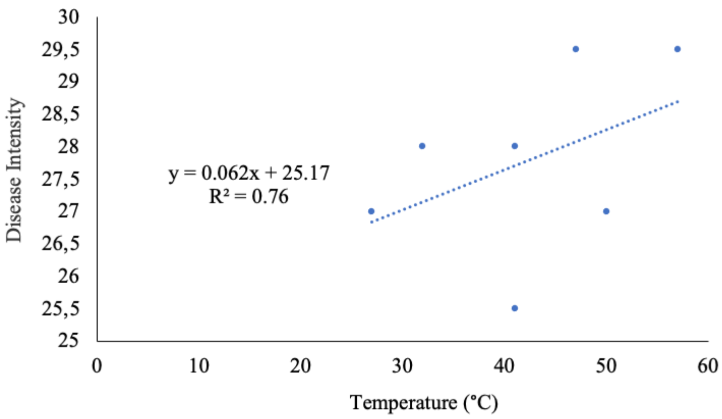


Figure 4. Regression Relationship between Temperature and Intensity of Disease

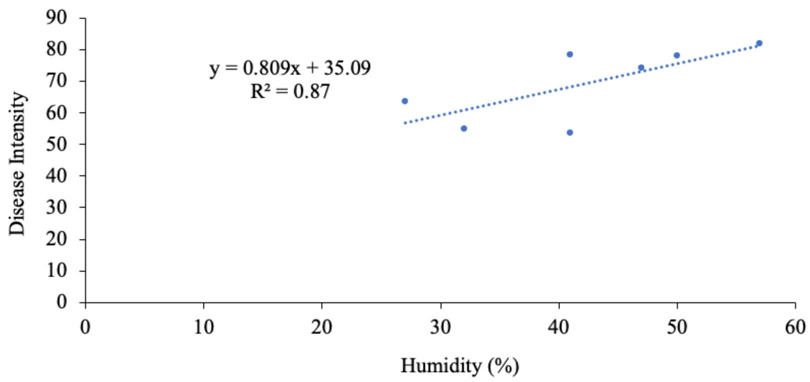


Figure 5. Regression Relationship between Humidity and Intensity of Disease

The observations and calculations revealed that a relationship between temperature and humidity factors on disease development factors was found. Based on the regression results it could be seen that the humidity and temperature factors were very dominant and a significant relationship with disease development factors shown by regression equation Y 0.062 X + 25.17 that indicated the relationship between temperature and intensity of the disease. The equation Y 0.8097 X + 35.09 showed the relationship between humidity and intensity of the disease. The results of this regression were used to predict the development of leaf rust disease in maize plants. The results of the two regressions, an R2 value of 0.76 that indicated the relationship between temperature and disease intensity, and R2 of 0.87 that indicated the relationship of humidity and disease intensity, were significantly different.

Based on Figure 4, the average temperature for 3 months of observation was 29.12$^0$C, while at first the emergence of leaf rust symptomatic plants was marked by the emergence of pustules occurred when the average daily temperature reached 27$^0$C with an average daily humidity of 64$^0$C totaling 4 plants from 24 plant samples in Bayur field. In Muang Land, at first the emergence of leaf rust symptomatic plants was also marked by the appearance of pustules occurring when the average daily temperature reached 27$^0$C with the average daily

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humidity of $78^0$C totaling 4 plants from 20 plant samples. High average humidity leveled up to > 80%, which contributed to the appearance of leaf rust.

## CONCLUSIONS

Based on the study, it is found that the fungus causing leaf rust disease in corn is *P. sorghi* Schw. The average rate of rust infection between Bayur Village and Muang Dalam Village, Lempake District was not different. Temperature and moisture play a role in the development of leaf rust diseases, but humidity plays a more important role in the development of leaf rust diseases on corn. The higher the temperature accompanied by the increased humidity, the more leaf rust disease develops. The soil fertility also influences disease progression. The nutrient-poor soils at two research sites cause leaf rust disease to develop well.

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In the results and discussion, there is no mention o data or statistical analysis that supports this statement.

## ACKNOWLEDGEMENTS

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**Comment [A32]:** The author cites 29 referenc we recommend using at least 35 references. Pleas add more explanation related references to compa and discuss with previous findings especially fron internationally reputed journals.
Please ensure that every reference cited in the text also present in the reference list (and vice versa).

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These references may be relevance to the paper:

Integrated disease management strategy of comm rust of maize incited by Puccinia sorghi Schw.; https://academicjournals.org/article/article143341 4_Dey%20et%20al.pdf

Reaction of some maize (Zea mays l.) varieties to infestation with root-knot nematode, Meloidogyn incognita under field conditions; http://www.academicjournals.org/app/webroot/art /article1379944999_Adegbite.pdf

Spatiotemporal yield loss assessment in corn due common rust caused by Puccinia sorghi Schw.; https://www3.iupui.academicjournals.org/article/a cle1380885953_Dey%20et%20al.pdf

Status of grey leaf spot disease in Kenyan maize production ecosystems; https://doi.org/10.4314/acsj.v18i4.68647

Infuence of weather factors on severity of aerial blight of soybean; https://doi.org/10.1007/s42360-020-00235-w

Correlation of stomata density to rust severity on some accessions of maize germplasm; http://jhpttropika.fp.unila.ac.id/index.php/jhpttrop /article/view/527

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**Field Code Changed**

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