CORRELATION OF VARIOUS ENVIRONMENTAL FACTORS TO COFFEE LEAF RUST DISEASE AND ITS NATURAL CONTROL

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ABSTRACT

Climate is an important factor in the development of plant diseases. to be able to avoid yield loss due to disease explosion, it is necessary to conduct an in-depth study of climatic factors as a direct and indirect influence. one that can be done is weather data-based forecasting. This method can determine and estimate the likelihood of disease outbreaks in a certain period. In addition, disease forecasting can act as prevention due to pathogen attacks so as to avoid damage and yield loss and maintain the ecosystem of antagonistic fungi in the coffee ecosystem. Agro ecosystem management can encourage the development of antagonistic fungi as pathogen biological agents. *Akanthomyces* sp. species are widely reported as entomopathogenic fungi. In Indonesia, *Akanthomyces* sp. was found to be associated with Arabica coffee leaf rust disease. This study was conducted in seven coffee plantation locations in Kalibening District, Banjarnegara Regency, Indonesia. This study detected environmental factors affecting the development of *Hemileia vastatrix*, predicted its population and determined the antagonistic fungus associated with *H. vastatrix*, namely *Akanthomyces* sp. TBK2, by molecular detection.

Keywords: Arabica coffee, climate, coffee leaf rust, *Akanthomyces* sp, *Hemileia vastatrix*

الارتباط بين عوامل البيئة المختلفة ومرض صدأ الاوراق والسيطرة الطبيعية عليه

نور خليدة	نور بريهاتينينجسيه	إندانغ موغياستوتي	أجوس ريانتو	سيتي نورشاسانا
باحثة	استاذ	باحثة	باحث	باحثة

المستخلص

يعد المناخ عاملاً مهماً في تطور الأمراض النباتية، ولتفادي خسارة المحصول بسبب تفشي الأمراض، من الضروري إجراء دراسة متعمقة للعوامل المناخية كتأثير مباشر وغير مباشر، ومن بين الطرق التي يمكن القيام بها هي التنبؤ القائم على بيانات الطقس .يمكن لهذه الطريقة تحديد وتقدير احتمالية تفشي الأمراض في فترة معينة .بالإضافة إلى ذلك، يمكن للتنبؤ بالأمراض أن يعمل كوسيلة وقاية بسبب هجمات مسببات الأمراض لتجنب الأضرار وفقدان المحصول والحفاظ على النظام البيئي للفطريات المضادة في النظام البيئي للقهوة، حيث يمكن لإدارة النظام البيئي الزراعي أن تشجع على تطوير الفطريات المضادة كعوامل بيولوجية ممرضة .وقد تم الإبلاغ على نطاق واسع عن أنواع Akanthomyces sp. في إندونيسيا، وُجد أن فطر Akanthomyces sp. مرتبط بمرض صدأ أوراق البن العربي .أُجريت هذه الدراسة في سبعة مواقع لمزارع البن في منطقة كاليبينينغ، محافظة بانجارنيجارا بإندونيسيا .كشفت هذه الدراسة عن العوامل البيئية التي تؤثر على تطور الهيميليا المتوسعة، وتنبأت بتعدادها وحددت الفطريات المضادة المرتبطة بمرض صدأ أوراق البن العربي وهي أكانثوميسيسيس سب . TBK2 عن طريق الكشف الجزيئي.

الكلمات المفتاحية :قهوة أرابيكا، المناخ، صدأ أوراق القهوة، *Akanthomyces sp*، *Hemileia vastatrix*.

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INTRODUCTION

The increase in global temperatures in various parts of the world is a clear indication of changes related to climate change issues. The Intergovermental Panel on Climate Change (IPCC) states that the Earth's temperature has increased by about 0.80C over the last century. This means that the temperature has gotten much hotter and will continue to warm, otherwise known as “Global Warming”. In addition to an increase in the Earth's temperature, the effects of global warming have also led to the frequency of heat waves and the intensity of rainfall in various parts of the world. One of the impacts of climate change on coffee plantations in Indonesia is the development of rust disease. Based on the results of research in 2023, data obtained that the main disease that damages coffee plantations is leaf rust disease (25). Coffee is a plantation crop commodity that has an important role in supporting the country's economy (4). The coffee industry has contributed as a driver of coffee farmers' income, a source of foreign exchange, a producer of industrial raw materials, and a provider of employment opportunities through processing, marketing, and export and import trade activities. The socio-economic development of society causes the demand for coffee to continue to increase. The growth of coffee retailers and roasters has caused demand for coffee beans to increase in almost all regions of Indonesia and even around the world. Demand growth is strongly influenced by coffee quality and world demand (22). Improving coffee quality can be done by fertilizing (21) and managing the main disease, namely coffee leaf rust. *Hemileia vastarix* attacks produce leaf rust disease, which disrupts plants by causing light yellow to dark yellow patches on the tops of the leaves. Uredospores are produced as the stain progressively grows larger. Yellowish-green, chlorotic patches devoid of sporulation are the symptoms. Plants that have advanced infections of leaf rust lose their leaves and get bald. Climate change mitigation in dealing with disease development is necessary. Conventional control of coffee leaf rust disease is not effective. Conventional disease control is disease control using chemical pesticides. The continuous use of chemical pesticides in agricultural systems can cause resistance to pathogens so that new pathogen species will emerge so that pathogen populations cannot be controlled. Through natural management can allow the antagonistic ecosystem to grow well. Weather data-based forecasting or epidemiology is a breakthrough in monitoring and forecasting the potential spread of plant diseases. This method can estimate the possibility of a disease explosion in a certain period. In addition, disease forecasting can act as prevention due to pathogen attacks so as to avoid damage and yield losses and maintain the ecosystem of antagonistic fungi in the coffee ecosystem. Proper crop management will support sustainable agriculture in the future. The mechanisms of antagonistic fungi of *H. vastatrix* are hyperparasitism, mycoparasitism, and fumicolus fungi (3). Hyperparasitism is a condition where fungi are parasitic on other fungi. Fungi can parasitize other organisms and have negative impacts on their hosts, including changing virulence to the point of causing death (14). There have been many studies on H. vastatrix management, but only a few have focused on the Arabica coffee ecosystem in the Dieng Mountains, Indonesia (25). This study aims to determine the factors affecting the development of *H. vastatrix*, predict its potential spread, and the presence of biological control agents in the Arabica coffee ecosystem of the Dieng mountains through molecular investigations.

MATERIAL AND METHODS

Study area and samples: The Dieng Mountains are located at 1,600-2,600 meters above sea level in Central Java Province, Indonesia. The research was conducted from April 2023 to July 2024. Samples were collected by the research team in collaboration with Banjarnegara District Field Extension Officers.

Measuring Weather Data: Measuring weather data using *Internet of Things* (IoT) *technology* involves using sensors connected to a network to monitor and collect information about atmospheric conditions and the surrounding environment. Weather sensors used include thermometers, thermohygrometers, and anemometers. The weather sensors used are installed in predetermined locations that are in the area under the canopy so that they can determine the exact microclimate. The data captured by the sensor is then configured to send data from the weather sensor to the server. Data capture can be done with software and converted into a format that can be interpreted and processed. Sensor application is done before the observation of disease severity. The sensor will provide data on temperature, humidity, and wind speed so that environmental factors in the development of coffee leaf rust disease will be known.

Measuring The Distribution Rate of Coffee Leaf Rust Disease: The research took the form of a survey using the Purposive Random Sampling method to determine the research location. The criteria for site selection were plantations in the Arabica coffee production center area in the Dieng mountains, Banjarnegara Regency with the criteria of productive plants aged 4-12 years. The locations were located in 4 sub-districts of Kalibening, Pejawaran Pagentan, and Wanayasa. Furthermore, at each location a village point was selected for sampling. The total land for observation was 14 plots. The selected sample points are areas with different geographical conditions. Different geographical conditions will affect the spread of the disease, so that decisions in preventing epidemics can be made appropriately. Distribution measurement is done by mapping diseased plants and healthy plants.

Measuring The Severity of Coffee Leaf Rust Disease

The sampling method used consisted of 14 sampling plots in an area the size of each location with a minimum area of 1750m2. Each sampling location was numbered and placed on a map, following the plot location in the field to facilitate its location. At each location point, 14 coffee plants were randomly selected and from each plant ten leaves were taken from the bottom, middle and top of the plant using four cardinal points (north, south, east and west) (Figure 4). Based on the sample points obtained, the disease severity was measured. Measurements were made using the Purposive Random Sampling method to determine the location of the study. Sampling is done randomly by setting diagonal points as samples. Furthermore, it is calculated and analyzed according to the disease severity value.

$IS = (n \div N) \times 100\%$

Attack intensity values are calculated using the following formula:

Description:

IS = intensity of attack (%)

n = number of plant samples or specific plant parts that are absolutely damaged

N = number of plant samples observed

Symptoms of Arabica coffee plants infected with rust disease are classified as follows:

Table 1. Coffee disease scores and criteria

Score	Criteria
0	No symptoms
1	Mild symptoms 20%
2	Moderate symptoms 40%
3	Widespread symptoms 80%
4	Plant death

DNA extraction: DNA extraction of the Akanthomyces fungus was carried out using the QuickDNA fungal miniprep kit (26). This method was also previously used by (25). Determining whether genera or species share molecular homologies or similarities is the goal.

Morphological and molecular identification

Morphological identification- *Akanthomyces* sp. fungus isolated from Arabica coffee leaf rust colonies was purified by growing on PDA media. Based on the results of morphological observations, it was identified as *Akanthomyces* sp. (Figure 1). *Molecular identification*- Primer ITS is one of the universal primers used in PCR analysis (Figure 2). BLASTN searches were used to search for sequences from various putatively divergent regions. Sequences—*Phylogenetic Analyses*- Are DNA concentration measurements made with the Gene Spec Instrument. The TaKaRa PCR Thermal Cycler is used to carry out DNA doubling, sometimes referred to as the sequence cycle process. Ten seconds of setting, five seconds of annealing at 50°C, four minutes of extension at 60°C, and twenty-five repetitions were completed. At Genetics Science in Serpong, Tangerang, Banten, samples were examined.

RESULT AND DISCUSSION

Based on the observations, data were obtained including coffee leaf rust disease intensity, wind speed, temperature, air humidity (Table 1).

Table 1. Observation data of intensity & microclimate

Wind speed (m/s)	Temperature (°C)	Air humidity (%)	Disease Intensity (%)
0,9	18,4	92,3	11,55
1,1	18,7	90,2	11,7
1,5	19,8	93,9	12,11
2,7	19,9	95,3	13,49
3	18,5	89	13,77

The lower the temperature and humidity, the higher the disease intensity. However, conversely, disease intensity will increase if light intensity and wind speed increase. Furthermore, correlation analysis was conducted to determine the closeness of the relationship and the direction of the relationship of each variable Table 2. Measuring the severity of coffee leaf rust disease Activities were carried out using the random sampling method. Disease symptoms found in the field were then identified in the laboratory to confirm the type of pathogen that attacked. Furthermore, the pathogens found were tested for pathogenicity to determine virulence against plants.

Table 2: Microclimate correlation to disease intensity

Correlations		Wind speed	Temperature	Air humidity	Disease Intensity
Wind speed	Pearson Correlation	1	.254	-.024	.999**
	Sig. (2-tailed)		.680	.969	.000
	N	5	5	5	5
Temperature	Pearson Correlation	.254	1	.836	.247
	Sig. (2-tailed)	.680		.078	.689
	N	5	5	5	5
Air humidity	Pearson Correlation	-.024	.836	1	-.014
	Sig. (2-tailed)	.969	.078		.983
	N	5	5	5	5
Disease Intensity	Pearson Correlation	.999**	.247	-.014	1
	Sig. (2-tailed)	.000	.689	.983	
	N	5	5	5	5

Based on the results of the analysis conducted, the intensity of coffee leaf rust disease is influenced by wind speed. The impact of wind speed has an impact on increasing the intensity of leaf rust disease. Wind is one of the intermediaries for the spread of *H. vastatrix* pathogen spores (11). A slight breeze can cause plants around the inoculum to become infected with coffee leaf rust disease (7). The transfer of spores to a new host will initiate a cycle of infection to healthy and diseased plants, thus increasing the spread and intensity of the disease. Plant disease development is influenced by a variety of complex environmental factors, including air temperature and humidity. Air temperature plays an important role in pathogen infection, infection rate, latency period, sporulation, inoculum dispersal, and affects host resistance. Low air temperature with high humidity levels can form dew and be used by pathogens as a water source (9). Humidity has little effect on rust disease development. Observation data showed that the humidity of the coffee agroecosystem averaged 90%. With this humidity, *H. vastatrix* can germinate and plus dark and wet conditions for 6-8 hours (19). The observed temperature and humidity variables influence each other but form an indirect effect on the intensity of coffee leaf rust disease. The research was carried out through field exploration in the Arabica coffee ecosystem, a geographical indication of Dieng, Indonesia. Several samples obtained showed morphological characteristics, namely oval-shaped spores and non-separated. The isolate used in the genetic study showed that the identified TBK2 DNA had a DNA band measuring 581bp, belonging to the genus *Akanthomyces*. *Akanthomyces* is an antagonistic fungus that lives in the phyllosphere and infects various pathogens. The range of pathogenic fungi includes *Fusarium verticilloides*, *F. solani, F. oxysporum, Fusarium* sp., and *Curvularia lunata* (18). *A. muscarius* produces extracellular enzymes that can penetrate the host cuticle (1). *Akanthomyces* sp. has been confirmed to have a detrimental effect on the growth of *Hemileia broadratix* (3). Apart from having a negative impact on pathogens, *Akanthomyces* has been identified as an entomopathogenic fungus that can infect the insect pest *Megalurothrips usitatus* (Bagnall) and can be deadly at LT50 (4). The morphological characters of *Akanthomyces* sp TBK2 are mostly elliptical or oval (Figure 1), form chains, are not slimy (17), and are white to yellowish in color. Hyphae have smooth, branched, and insulated hyaline walls. Conidiophores are smooth-walled and circular in shape. Conidia are hyaline, single-celled, round, and 1.6–2.4 µm in diameter. Colonies on PDA grow rapidly at 250 °C (24). The detection reported in this study was specifically *Akanthomyces* sp. TBK2 found in *H. vastatrix* colonies in the Arabica coffee production center, Banjarnegara, Indonesia with a tropical climate. *Akanthomyces* sp. TBK2 was found to parasitize *H. vastatrix*, so it appeared that *Akanthomyces* sp. was actively growing in urediniospores and infecting cells. In a study (12) identified *A. lecani* was identified as an agent for controlling rust disease in peanuts caused by *Puccinia arachidis*, *Cercospora arachidicola*, and *Phaiosariopsis personata* in the Burkina, Faso region.

Figure 1. Morphology of *Akanthomyces* sp TBK2 isolated from Arabica coffee

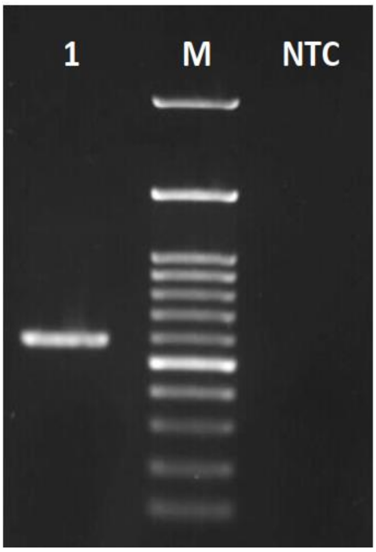

Figure 2. Analysis of PCR product by electrophoresis on 1% TBE agaroses, 1. Sample isolates of *Akanthomyces* isolates TBK2, M. DNA ladder, NTC. Nontemplate control.

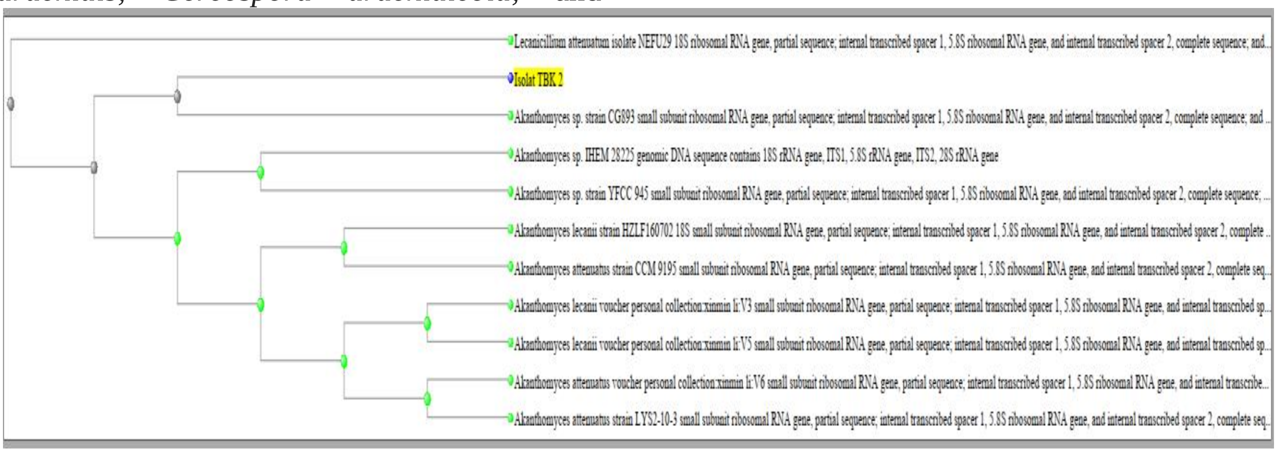

Figure 3. Based on the ITS sequencing, the phylogenetic tree of the isolates of *Akanthomyces* sp. TBK2 isolates. Yellow highlights indicate new isolates.

Akanthomyces sp. is a fungus with high diversity, having a wide distribution among natural microorganism habitats. Wang et al. (24) obtained four new species identified as *Akanthomyces*. These species include *A. kunmingensis*, *A. subaraneicola*, *A. laosensis*, and *A. pseudonoctuidarum*. *Akanthomyces* species were isolated from soil and invertebrates from China, Laos, and Thailand. The existence of antagonistic fungi is studied because it produces various mechanisms that can inhibit pathogens. *Penicillium chermesinum* produces toxic compounds in inhibiting the pathogens *Fusarium* sp and *Ralstonia solani* (15), produce secondary metabolites (2). However, research regarding the colonization of *Akanthomyces* sp. in parasitizing coffee rust disease has not been widely documented. This study provides information for the first time that cellular interactions between *Akanthomyces* sp. TBK2 and *H. vastatrix* occur in the Dieng Mountains, Indonesia. *Akanthomyces* sp. is found in the phyllosphere of Arabica coffee leaves in association with *H. vastatrix*. Nicoletti and Becchimanzi (13) stated that from their findings, *A. muscarius* was isolated as an endophytic microbe in healthy leaves of *Nypa fruticans* and as an endophytic microbe in Edelweiss (16). Research on the antagonistic mechanism of *A. lecani* against rust disease in peanuts was described by (12). There is at least one species that has been detected as an antagonistic fungus for coffee leaf rust, namely *Akanthomyces* sp. TBK2, however many species of *Akanthomyces* have been detected as entomopathogens, including *A. ovalongatus*, which infects spiders (17, 8), *A. noctuidaru*, *A. pyralidarum*, *A. tortricidarum* on months (1), *A. uredinophilus* on Hemiptera Insect (10), *A. gracillis* on Lepidoptera (20), *A. muscarius* a biocontrol agent of insects (6). Phylogenetic analysis of available isolates and sequences shows that *Akanthomyces* has a wide geographic and host range. Manfrino et al. (10) explain the distribution of *A. uredinophilus* in New Zealand, Colombia, USA, Kenya, Germany, Africa, Iran, China, and Mexico. and associated with plants such as *Coffea* sp., *Heliotropium peruvianum*, *Arachnida* sp., and *Sargassum* sp. (23). However, PCR sequencing obtained in this study is needed to determine the specific species involved in the biological control of Arabica coffee leaf rust disease *H. vastatrix* in Banjarnegara, Indonesia. Plant disease development is influenced by a variety of complex environmental factors, including air temperature and humidity. The intensity of coffee leaf rust disease in the Dieng Mountains of Banjarnegara Regency is influenced by wind speed. *Akanthomyces* sp. TBK2 was detected as a natural antagonistic fungus of Arabica coffee leaf rust disease in Banjarnegara, Indonesia. The species identity was confirmed as *Akanthomyces* sp. TBK2 by amplifying a specific ITS gene.

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