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RESEARCH PAPER

Bioecology of rust on *Dendranthema grandiflora* in Yogyakarta-Indonesia

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ABSTRACT

Rust leaf disease is the main disease on chrysanthemum (*Dendranthema grandiflora*) plantations in the district of Sleman, Yogyakarta Special Region. In 2006, the symptoms of leaf rust were initially found in chrysanthemum farms in village of Hargobinangun and surrounding areas. Since then it has been consistently spreading to other areas throughout Yogyakarta-Indonesia. *Puccinia horiana* is closely associated with rust leaf disease. This research was aimed to investigate the distribution, incidence, and severity of chrysanthemum rust disease in chrysanthemum plantations in Yogyakarta. The survey was conducted based on a randomized stratified approach. The entire district was divided into five observation sites, with each location consisting of three to four chrysanthemum farm sites owned by farmers. The results showed that rust leaf disease was distributed in all sites. The average of disease incidence found in the observation sites was 97.13%, meanwhile the average of disease severity was 55.19%. The highest disease incidence was found in Kaliurang 98.44%, followed by Wonokerso of 98.33%, Gondanglegi of 96.87%, Boyong of 96.25%, and Sidorejo of 95.83%. The highest disease severity was found in Wonokerso 58.75%, followed by Gondanglegi 56.92%, Kaliurang 53.39%, Sidorejo 54.89%, and Boyong 52.01%. The lower plant cultivation site, the lower incidence of rust disease.

Key words: disease distribution, disease incidence, disease severity, leaf rust disease

INTRODUCTION

Rust leaf disease is the most serious disease affecting chrysanthemum (*Dendranthema grandiflora*) in Yogyakarta Special Region, Indonesia. Yogyakarta is one of the central chrysanthemum plantations in Indonesia. *Puccinia horiana* is the causal agent of chrysanthemum white rust or familiar called *Japanese Rust*. This microcyclic autoecious rust has a quarantine status and can cause major damage in the commercial production of *Chrysanthemum x morifolium*, or now called *D. grandiflora*. Given the international and often trans-continental production of planting material and cut flowers of chrysanthemum and the decreasing availability of registered fungicides in specific regions, breeding for resistance against *P. horiana* will gain importance and will need to involve the appropriate resistance genes for the pathotypes that may be present (de Backer et al*.,* 2011).

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Rust disease was first reported by Team from The Assessment Institute of Agricultural Technology, Yogyakarta, Indonesia in 2006. Initially, the symptoms of leaf rust were found in chrysanthemum farms in Hargobinangun District and surrounding areas. Then in extended observation during July 2007, it has spread to all chrysanthemum plantation areas in Sleman Regency: Pakem, Cangkringan, Ngemplak, Turi. It indicated that rust disease was already causing crop damaged throughout the major chrysanthemum growing areas while producing around 8000–10,000 stem of chrysanthemums every big harvest (Masyhudi & Suhardi, 2009). It was found that around 50% of the cultivar variety of Sakuntala showed leaf rust symptoms, while only 3% of Puspita Nusantara and Regent cultivar varieties (Martini et al., 2015).

Chrysanthemum leaf disease caused by the fungus *Puccinia horiana* was reported. This pathogen was found on chrysanthemums showing rust disease symptoms (Hanudin et al., 2011). Based on many years of research, it was suspected that a pathogen was the cause of rust disease, but the identification of the pathogen was inconclusive. Symptoms were visible 7–10 days after initial infection followed by the production of telia. Symptoms of CWR were characterized by the presence of pale-green to yellow-white rust spots (5 mm diameter), on the upper surface of the leaf. The Centre of the spots turns brown and necrotic upon aging. On the corresponding lower surface, raised, buff, or pinkish, waxy pustules (telia) were observed. Severely affected leaves wilt, droop and gradually dry up (Dheepa et al*.,* 2016).

During surveys in September 2010 and December 2012, samples from symptomatic tissues were obtained, especially those associated with leaf and stem damage. The results showed that spora (teliospore) was commonly isolated in the leaf of *D. grandiflora* and consistently isolated from the stem. Chrysanthemum pathogenicity tests were conducted on a range of fungal organisms isolated from diseased tissues. Tentatively identified based only on morphological characteristics revealed that fungus *P. horiana* is the causal agent of chrysanthemum yield decline. Artificial inoculation on chrysanthemum saplings in the greenhouse and on mature trees more than 3 weeks in the field produced extensive lesions, similar to those found on the seed of chrysanthemum mother plants. Although infection of this disease occurs mainly on leaves caused by the seed (cutting stem), it was also shown to occur on mechanical and airborne. It was observed that this disease can be potentially spread by all mechanical inhabiting, while the progress of the pathogen within each tree is facilitated greatly by the extensive spread of the airborne within the wrong cultivation (Martini et al., 2009).

Studies to evaluate the role of cutting as an inoculum source, variety resistance, and fungicide application interval were done at the Indonesian Ornamental Crops Research Institute. Survey of cutting health was carried out at farmer's fields as seed producer in July 2002. A study under the plastic house to evaluate the response of some cultivars and determine the efficacy of fungicide applications was carried out from July–September 2002. The results indicated that cuttings were the inoculum source of rust on the chrysanthemum. On the individual plant, both under plastic and glasshouse, the development of white rust was suppressed (Suhardi, 2009).

The objective of this research was to study the distribution, incidence, and severity of rust leaf disease in chrysanthemum plantations in Special Region of Yogyakarta Province, Indonesia.

MATERIALS AND METHODS

Research Site. The research was conducted in Hargobinangun, belongs to the sub district of Pakem, Sleman District, located at an altitude ranging from 500–1325 masl, or belongs to the category of medium to high plains and has an average temperature of $\pm$26 °C. Types of regosol soil with flat topographic regions up to 40% marbled and moderate fertility rates. Here's a map of the observation of the distribution, incidence, and severity of rust leaf disease in chrysanthemum plantations in Special Region of Yogyakarta Province, Indonesia. The location is on a stretch from 107°15'03" to 100°29'30" East Longitude and 7°34'51" to 7°47'03" South Latitude (Figure 1).

Surveys were conducted based on a stratified random approach. Five hamlets where the chrysanthemum farms were exist were chosen. Three to four chrysanthemum farms were then determined in each hamlets as observation areas (Table 1).

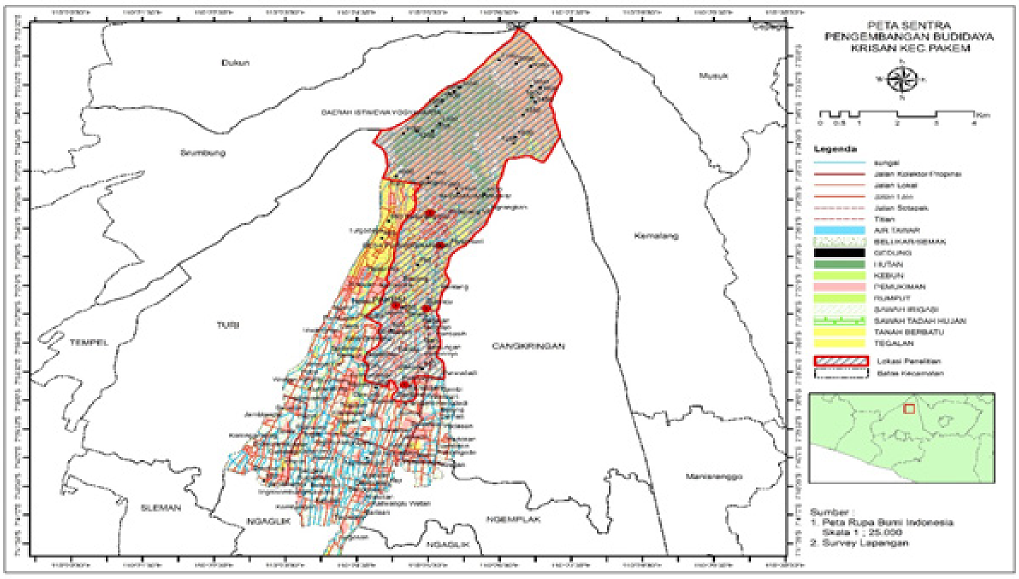

Figure 1. Physiography Map of Pakem, Sleman District.

Observation on the Air Borne Spore of *P. horiana*. Observation of spore transfer was done by catching spores for 12 months, which is divided into 2 seasons, namely when the rainy season changes to the dry season, and vice versa as well as when the dry season changes to the rainy season. Spore observations are made every 2, 4, and 12 h. Observation of the collected spores were made at the chrysanthemum cultivation center in District of Sleman, Special Region of Yogyakarta Province.

Disease incidence and disease severity caused by *P. horina*. The disease incidence was estimate according to the formula:

$$DI = \left(\frac{n}{N}\right) \times 100\%$$

DI = Disease Incidence (%);
n = Number of chrysanthemum trees showing leaf rust symptoms;
N = Total number of chrysanthemum trees observed.

The disease severity was calculated using formula:

$$DS = \left[\Sigma \frac{(n \times v)}{(N \times V)}\right] \times 100\%$$

DS = Disease Severity (%);
n = Number of chrysanthemum trees of same score;
v = Score of each symptom category;
N = Number of chrysanthemum trees observed;
V = The highest score of symptom category.

The leaf symptom catergory was shown in Table 2.

RESULTS AND DISCUSSION

Symptom of *P. horiana* on chrysanthemum. Rust is the most serious disease in chrysanthemum plants because heavily infected plants cannot be sold causing harm. On chrysanthemum planting in Asia, *P. Horiana* is an *autoecious* rust pathogen (can complete its life cycle in a single host). Infection is characterized by yellow patches above the surface of the leaves, which can further cause necrotic symptoms. *P. horiana* produces two types of spores, teliospores, and basidiospores in appropriate environmental conditions, which are produced at the bottom of the leaf surface (Wise et al., 2004). Figure 2 shows the symptoms of rust disease in chrysanthemum

Table 1. Location of chrysanthemum planting based on altitude

Hamlets where the chrysanthemum farms were exist	Altitude (meter above sea level)	Village
Sidorejo	500 – 600	
Wonokerso	450 – 550	
Kaliurang	850 – 1000	Hargobinangun
Boyong	700 – 850	
Gondanglegi	380 – 550	

Table 2. Scoring plants based on the severity of white rust disease symptoms

Score	Symptom category
0	0–25% of the chrysanthemum tree canopy affected
1	more than 25–50% of the chrysanthemum tree canopy affected
2	more than 50–75% of the chrysanthemum tree canopy affected
3	more than 75% of the chrysanthemum tree canopy affected

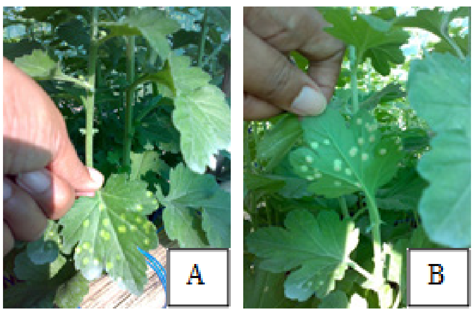

Figure 2. Symptoms of rust disease. (A) appear from the top surface of the leaves; (B) appear from under the leaves (Martini, 2014).

plants planted in rice fields in Pakem District, Sleman Regency. Symptoms of rust disease begin to appear in 25-day-old plants. Common symptoms of rust disease are white nodules on the lower leaves or pale deep grooves on the upper leaf surface. The spread of the disease can occur when farmers walk among sick plants and then move to healthy crops (Garcia & Topinka, 2018). Therefore The Food Inspection Agency (CFIA, 2019) recommends that the anticipation of the spread of disease is done by disinfecting clothes, hands, and shoes, before entering into the ranks of healthy plants.

Air Borne Spore of *P. horiana*. The results showed that teliospores were caught at each installation time. Teliospores were mostly observed at 10:00 am–02:00 pm and 02:00 pm–06:00 pm (West Indonesian time). It has been reported that it is difficult to make observations on the teliospore in nature (at the chrysanthemum cultivation center in Sleman Regency, Special Region of Yogyakarta Province).

At the time of releasing basidiospore, germination, and infection take minimum 5 h at optimum relative humidity of 96% and temperature between 17–24 °C. Desprez-Loustau & Dupuis (1992) stated that the germination of *Melampsora pinitorqua* teliospores was observed throughout their natural activation period during the spring. The spread of basidiospores turns out to have the ability to germinate. It is monitored in the field and proven to be controlled by the activation stage of teliospore, as well as occurring during rainfall. Teliospore germination follows a bell-shaped curve over time. Optimal germination corresponds to the maximum amount of basidiospores produced and at the maximum speed of production and can be used to predict the beginning of the spread of basidiospores in the field and the level of germination of low teliospores *M. pinitorqua* (Desprez-Loustau & Dupuis, 1992).

Disease Incidence and Disease Severity. Totally 17 chrysanthemum farms were found in the 4 observation sites. The farm has a field area between 100 to 200 m^2 with a total population of 150.000 plants. The highest incidence (100%) of rust leaf disease found in Kaliurang (-7.592126555958181, 110.42533079702699), with disease severity was 58.43% (Table 3).

Using large-scale overlay models for pathogen-spreading systems, our experimental data showed that the chamber did not limit the movement of weather-dispersed plant pathogens. Connectivity between overlays and disease rates did not increase plants infected with foliar fungi. From the field, known edge effects are the main drivers of plant disease dynamics. The increased spread of infectious diseases is often cited as a potential negative effect of habitat corridors used in conservation, but the impact of corridors on the movement of pathogens has never been empirically tested. Variations in average daytime temperatures provide a possible mechanism for the pattern of the disease (Whipps, 1993). Our results suggest that concerns over the potentially harmful effects of conservation corridors on disease dynamics are misplaced, and that, in the context of conservation, many diseases can be better managed by reducing the effects of edges. Using sweet corn (*Zea mays*) and southern corn leaf disease (*Cochliobolus heterostrophus*) as models of plant-pathogen systems, the impact of connectivity and habitat fragmentation on pathogen movement and disease development has been tested at the Savannah River Site, South Carolina, USA. Over time, less strained patches have a higher proportion of plants, and the distance of the host plant to the edge of the habitat is the biggest determinant of disease development (Johnson & Haddad, 2011)

The development and spread of fungi can be interpreted similarly to mimic the aromatic properties of some flowering plants and the appearance of a beautiful body as if fruiting of fungal. Besides, infection of plants by certain fungi can direct plants to develop nonfunctional flower-like structures that remain primarily serving the advantages of fungal reproduction. This variety of mimicry can serve to attract insects that in turn spread fungal spores or plant pollen, thereby facilitating the sexual reproduction of crypto organisms (Kaiser, 2006).

From Figure 3 it appears that the spores from the capture are identical to the spores of *P. horiana*. This step is the first step to analyze the results of the captured spores. The potential yield loss caused by these diseases depends on host susceptibility and weather conditions, but the loss also is influenced by the timing and severity of disease outbreaks relative to the crop growth stage. The greatest yield losses occur when one or more of these diseases occur before the heading stage of development. Early detection and proper identification are critical to in-season disease management and future variety selection.

Since rust leaf disease was first reported in 2006 in Hargobinangun and surrounding areas, it has spread to other chrysanthemum plantations very fast. Currently, rust disease is distributed throughout all chrysanthemum plantation areas in Sleman (altitude range 80–780 masl). The highest disease incidence (100%) was found in Hargobinangun, where the symptoms of rust disease were first reported. In chrysanthemum plantations in

Table 3. Disease incidence of rust leaf disease in Each Site

Site / Location of chrysanthemum farm	Ordinate	Disease Incidence (%)	Average (%)	Disease severity (%)	Average (%)
Kaliurang	-7.5947639742272335, 110.42781988689521	98.75	98.44	51.56	53.39
	-7.592126555958181, 110.42533079702699	100		58.43	
	-7.597061067272604, 110.42335669126943	97.50		56.56	
	-7.597869300793434, 110.42623201922065	97.50		55.00	
Wonokerso	-7.629118065626886, 110.41877577588642	98.75	98.33	60.31	58.75
	-7.629458349214042, 110.4194624213673	98.75		58.12	
	-7.631159763088907, 110.4194624213673	97.50		57.81	
Gondanglegi	-7.629118065626886, 110.41877577588642	98.75	96.87	57,82	56.92
	-7.629458349214042, 110.4194624213673	95.00		55,12	
	-7.631159763088907, 110.4194624213673	96.25		51,23	
	-7.629118065626886, 110.41877577588642	97.50		56,00	
Boyong	-7.6243356449481725, 110.4197002258131	95.00	96.25	54.18	52.01
	-7.626175320430705, 110.41958220862107	96.25		48.43	
	-7.627153641743596, 110.4195071067716	97.50		53.43	
Sidorejo	-7.628167992387041, 110.42524350464878	92.50	95.83	54.27	54.89
	-7.628306232933993, 110.42521131814185	98.75		55.10	
	-7.628316866820362, 110.42537225067643	96.25			
Average			97.13		55.19

surrounding areas of Hargobinangun, and other areas the disease incidence varied from 92.50–98.75%, and the average in all sites 97.13%. In 1990 it was reported that rust disease infected the second generation of chrysanthemum trees because the previous generation (mother plants) were all dead due to this disease.

Disease severity varied from 52.01–58.75%, the highest in Wonokerso, and the lowest in Boyong. It was found that in sites where the chrysanthemum trees were grown in fertile soil and farmers used fertilizer, disease severity was not so high. The application of fertilizer appeared to increase the defense of chrysanthemum to rust disease. Some problems were previously implicated as the cause of rust disease namely, soil fertility, chrysanthemum variety, environmental factors, pests, and disease (Martini et al., 2009).

The relationship between the two measurements of plant diseases, incidence, and severity form an

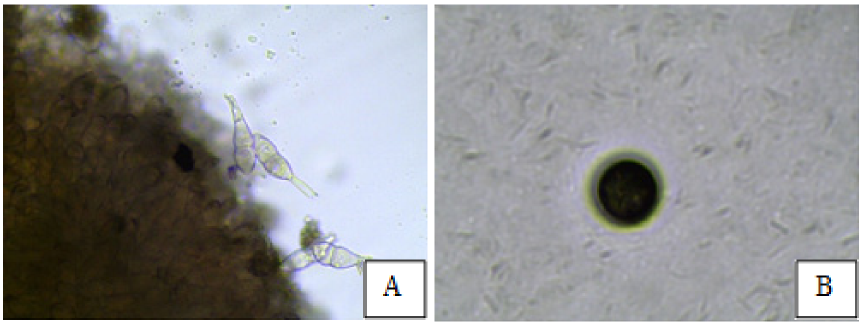

Figure 3. *Puccinia horiana* spores. (A) *P. horiana* which were scraped from the leaves of a sick chrysanthemum; (B) *P. horiana* is caught on the glass of an object mounted on a spore catcher (Martini, 2014).

epidemiologically significant concept whose potential has never been fully realized. Some researchers have recognized that because incidents are easier to measure than severity, any measurable relationship between the two steps would allow for estimation of severity based on incident data, which is easier to obtain and more precise (Seem, 1984).

Managing unstable season factors in medium plains with relatively high temperatures is a major challenge for farmers. Better ways are needed to monitor plant growth and diagnose the causes of poor plant growth. To improve cultivation management until the harvest period, the relationship effect between biotic and abiotic factors must be integrated and evaluated as an integrated system. Based on our research, information about season factors with relatively stable day and night temperatures is useful in identifying cultivation management, especially pest and disease management, through the application of water and fertilizers. Water and fertilizer management must be equipped with environmental management at certain elevations, especially for the cultivation of chrysanthemum in the medium plains.

Isolation of pathogens from symptomatic chrysanthemum tissues, especially those from the pustules of leaf *P. horiana*, showed that the spora (uredospores) of fungus was commonly found (Martini, 2014). Identification, based on morphology, showed that a new species *P. horiana* was associated with the leaf of chrysanthemums on Yogyakarta, and the cutting stem also airborne has a very broad host range, including crops and horticultural plants. *Ceratocystis ulmi* causes Dutch elm disease which is spread by bark-boring beetles, especially *Scolytus multistriatus* and *Hylurgopinus rufipes* (Mehrotra, 1980). *C. fimbriata* causes Mouldy rot on *Hevea* which is spread by insects and wind, as well as inoculated by knife wounds on rubber trees (Semangun, 1988).

It is known that the relationship between cutting rods for cuttings production is influenced by the climate and the source of rust disease pathogens that delight in conditions of high air humidity. A humid climate with the presence of resizing on the stem is a favorable condition for pathogens to infect. *P. horiana* infection is proven to occur in a field independent of insect borer. Pathogenic spores are proven to be carried by airborne, mechanical inhabiting, and some animals (insects and mites) inhabit and forge inside borer galleries. It is also observed that not all trees affected by borer insects are infected by *P. horiana*. However, field observations showed that the progression of the disease inside the tree after the initial infection was greatly improved by cutting the trunk, mechanical inhabiting, airborne, and insect borer activity. In addition to stems caused by rust borer, this pathogenic infection was also shown to occur in plants that are old around chrysanthemum crops.

Fungicide application was less effective in controlling white rust development, yet gave a significant impact on the plant height and number of leaves of chrysanthemum plants. Cutting selection based on the visual observation on the presence or absence of white rust pustules symptom did not give significant differences in the further development of the disease. The symptomless cuttings were also infected with this fungal disease after the cuttings were planted under the plastic house (Yusuf et al., 2017).

The pathogen reproduces readily and abundantly, both sexually and asexually within infected hosts. This contributes to the rapid spread of spores by cutting the stem, airborne, climate, mechanical inhabiting, and insects found within the borer galleries. It has been known through epidemiological surveys and investigations that show that high concentrations of potentially harmful fungi including pathogens in plants occur at intensive and alarming frequencies in the environment, both indoors and outdoors, including offices, homes, factories, and farms. Some fungi can produce various allergenic substances, odorous chemicals, and toxic metabolites (mycotoxins) (Ahmed, 2015) including offices, homes, factories and farms. Some fungi can produce a variety of allergenic substances, odorous chemicals, and toxic metabolites (mycotoxins. Some mycotoxins bind to the surface of fungal spores; others can be found in spores. As they multiply and spread through spores, for pathogenic diseases rust chrysanthemums can cause harm with a broad spectrum and can lead to crop failure.

As a result of the easy spread of spores, discussions about rust disease are now beginning to be directed at the utilization of growth-resistant cultivars. It is known that there are two main types of plant resilience, namely systemic resistance, and resistance. Both have been used in developing cultivars resistant to rust disease. Resistance begins from the nursery, can be detected at the seedling stage, but usually provides a high degree of resistance to certain stages at all stages of growth. Its high level, and its easy incorporation into commercial cultivars due to it's simple heritage, makes all-stage resistance more attractive to breeding programs and its contribution to rust disease control. The common occurrence and high level of pathogenicity suggest that *P. horiana* is closely associated with the recent acceleration of chrysanthemum death. The fact that the fungus appears to be endemic in the area provides a caveat that it could also threaten chrysanthemum production elsewhere in the world. Further research on this disease is essential to establish disease management strategies.

CONCLUSION

Leaf rust disease can be found distributed in all research sites, a disease incidence of 97.13% and disease severity of 55.19%. The highest disease incidence found in Kaliurang region which was reached 98.44%, followed by wonokerso was 98.33%, Gondanglegi 96.87%, Boyong 96.25%, and Sidorejo 95.83%. The lower the elevation of the research site, the lower incidence of the disease was found. The highest disease severity was occurred in Wonokerso region by 58.75%, followed by Gondanglegi 56.92%, Kaliurang 53.39%, Sidorejo 54.89%, and Boyong 52.01%.

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AUTHORS' CONTRIBUTIONS

TM and BHS are the main contributors. Shd, PY, and Sdmj are our member contributors. TM and BHS planned experiments, designed research, conducted field trials, conducted spore capture, conducted data analysis, and prepared manuscripts. Shd and PY have verified in the field, provided the evaluation of the implementation of activities in the field, and provided input on script writing. Sdmj provides an evaluation of the implementation of activities in the field related to monitoring and evaluating the implementation of activities financed by the 2013 State Budget. The author provides feedback and comments on the flow of research, data analysis, and the interpretation and form of the manuscript. All authors have read and approved the final manuscript.

COMPETING INTEREST

The author states that there are no competing interests regarding the publication of this manuscript.

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