Wilt Disease Intensity of Pineapple Plants (*Ananas Comosus* L. Merr) (Pineapple Mealybug Wilt-Associated Virus (Pmwav)) in Sub-District of Samboja

Ni'matuljannah Akhsan[1*], Rusmala Dewi[2]

[1]*Plant Pest and Disease Laboratory, Faculty of Agriculture, Mulawarman University. Jl. Pasir Balengkong, Kampus Gn. Kelua, Samarinda 75119, East Kalimantan, Indonesia*
[2]*Plant Pest and Disease Laboratory, Faculty of Agriculture, Mulawarman University. Jl. Pasir Balengkong, Kampus Gn. Kelua, Samarinda 75119, East Kalimantan, Indonesia*

**Corresponding Author: sempajaku@gmail.com*

Received: May 28, 2024
Accepted: June 2, 2024
Available online: June 10, 2024

Abstract. *Pineapple is one of Indonesia's commodity fruits with high economic value. Disease in pineapple plants is one of the factors that can affect productivity in pineapple plants, one of which is wilt disease caused by a virus (closterovirus) characterized by red leaves. The research was conducted from February 2023 to May 2023 in Bukit Merdeka Village, Samboja District. The research used descriptive methods with observations of PMWaV disease symptoms, cultivation techniques, PMWaP vectors and secondary pathogenic fungi. Sampling of disease intensity was done by purposive sampling, which is the withdrawal of samples based on predetermined criteria. Calculations were carried out on 4 pineapple fields with different plant ages. The parameters observed were disease intensity, presence and identification of vectors and secondary pathogenic fungi. The results showed that the intensity of PMWaV wilt disease on pineapple plants in Samboja District was 81.16% for land 1, 92.3% land 2, 79% land 3 and 73% on land 4. The age of pineapple plants on the land did not affect the intensity of PMWaV wilt disease attack. The vector of PMWaV is Dysmicoccus bravipes, secondary diseases in pineapple plants attacked by PMWaV disease are fungi Fusarium sp1, Fusarium sp2. and Curvularia sp.*

Keywords : pineapple wilt disease, pineapple mealybug wilt-associated virus, Dysmicoccus bravipes

1. INTRODUCTION

Pineapples are an Indonesian trade commodity with high economic value. Demand for fruit exports is increasing, this can be seen from the export value of pineapples in Indonesia in 2014 reaching US$ 140.08 million. The main destination country for demand for Indonesian pineapple exports is the United States amounting to US$ 56.32 (Kementan 2015). Pineapple production in Indonesia increases every year, but this increase is disrupted by pathogen attacks. In the last 10 years, pineapple plants in East Kalimantan have been a plant that has given hope for improving the economy of its people, after the decline in production of bananas, peppers and dragon fruit due to disease attacks. There was an increase in production from 21,975.65 tons (2020) to 55,170.05 tons (2021), which is around 151.05%. The increase in pineapple planting area also includes Samboja District, where the production is marketed apart from East Kalimantan itself as well as Sulawesi, South Kalimantan and Surabaya. Fresh pineapple fruit from Samboja District is very popular with consumers, because it tastes very sweet. Apart from fresh form, pineapple preparations are also made such as jam, syrup, sweets and madumongso (Mariati *et al.,* 2023).

Based on a survey in 2022 in Samboja District, at several pineapple planting locations, symptoms of PMWaV wilt disease were found. The attacks became more widespread because farmers did not know at all that their plants were attacked by PMWaV wilt disease, the pathogen and the vector of the pathogen. This ignorance causes farmers to never take control measures, as a result there is a decrease in weight per pineapple fruit and it is suspected that there is a spread of seed-borne diseases. Plants that are attacked by PMWaV at an age approaching the generative phase can still produce but the fruit they produce is smaller, while plants that are attacked in the vegetative phase are unable to produce even until the plants suffer serious damage. The presence of secondary pathogens causes more severe damage, especially the destruction of pineapple leaves. From the description above, it is necessary to know how high the intensity of PMWaV wilt disease is in pineapple plants of different ages, identify secondary pathogens and types of PMWaV vectors found in Samboja District.

2. LITERATURE REVIEW AND HYPOTHESIS DEVELOPMENT

Pineapple plants are included in the group of fruit plants in the form of bushes (shrubs) and are annual plants, pineapple plants can reach 1m in height. Pineapple plants can bear fruit all year round. Pineapples continue their growth for years through new vegetative shoots that grow from the stem. The new branches that grow can later produce fruit (Cahyono 2012). The classification of pineapple plants is included in the kingdom; Plantae, division; Spermatophyta, Class; Monocotyledonae, Order; Farinose (Bromeliales), family; Bromiliaceae, Genus; Ananas, Species; Ananas comosus (L) Merr (Collins 1961).

Diseases in pineapple plants are one of the factors that can affect the productivity of pineapple plants. Pineapple disease can be controlled early if you know the symptoms and causes of the disease. Symptoms of pineapple plant disease are an indication that the plant is attacked by a pathogen. One of the diseases that is an obstacle in increasing pineapple production is the Pineapple Mealybug Wilt-associated Virus (PMWaV) wilt disease. Wilt disease in pineapple plants is caused by a virus (closterovirus). Wilt disease is characterized by symptoms of leaf color changing from yellow to red, death of leaf tips, curling of leaves and wilting of the plant (Sutrawani *et al.,* 2010).

Virus transmission occurs due to the presence of vectors that help the spread of viruses that cause disease in plants (Sether *et al.,* 2001). Mealybugs (Dysmicoccus brevipes) as a disease vector greatly influence the development of disease in plants (Juarsa 2005). PMWaV wilt disease occurs due to an association between mealybugs (D. brevipes) which carry the closterovirus virus. The spread of the vector can be aided by the wind blowing the nymphs, the migration of fire ants (*Solenopsis* sp,) which live in symbiosis with mealybugs (D. brevipes) and parasitic nematodes (Sether *et al*., 2001). Pineapple plants attacked by PMWaV can still produce fruit, but the fruit produced is smaller than normal and experiences premature maturity, so it can be said to be economically unproductive (Sipes *et al.*, 2002). The hypothesis developed is that the younger the plant is attacked by PMWaV disease, the higher the level of farmer losses.

3. RESEARCH METHODS

This research was carried out in February - May 2023, located in Samboja District. Identification of pathogenic fungi and vector identification is carried out at the Plant Pest and Disease Laboratory, Faculty of Agriculture, Unmul. The materials used are pineapple plant leaves that have disease symptoms, 1% sodium hypochlorite, distilled water, potato dextrose agar (PDA), plastic wrap, 70% alcohol, methylene blue and label paper. The tools used in this research are scales, camera, laminar air flow, autoclave, oven, microscope, optical lab camera and other laboratory glassware.

The research was carried out using a descriptive method by observing farmers' cultivation techniques, disease symptoms on plants of different ages, calculating PMWaV disease intensity, identifying the vector and identifying secondary disease pathogenic fungi associated with pineapple plants in Samboja District.

4. RESULT AND DISCUSSION

Cultivation Techniques

Pineapple cultivation at the research location is carried out in monoculture. At the beginning of planting, the land is cleared and a bed approximately 1 m^2 wide is made. In one bed, two rows of plants are made with a distance of 30 x 50 cm^2. Fertilization is carried out at the beginning of planting, the fertilizer used is Urea, NPK, Phonska fertilizer. The dosage used is as recommended or according to the availability of fertilizer at the cultivation location. Plants are allowed to grow until they bear fruit at least three times without any additional fertilization. The pineapple seeds used come from seed providers in Samboja. Some plants that are 16 months old are already producing, but fruit weight has decreased greatly. Pineapple Cultivation of pineapple in Samboja District, more details can be seen in the table below

Table 1. Pineapple Cultivation in Samboja

Condition	Land 1	Land 2	Land 3	Land 4
Plant Age	4 months	8 months	12 months	16 months
Sample land area	2,250 m^2	2,250 m^2	2,550 m^2	1,200 m2
Fertilizer	Urea, NPK, Phonska	Urea, NPK, Phonska	Urea, NPK, Phonska	Urea, NPK, Phonska
Plant spacing	30x50 cm2	30x50 cm2	30x50 cm2	30x50 cm2
Number of plants	15,000	15,000	17,000	8,000
Origin of seeds	Seed provider in Samboja	Seed provider in Samboja	Seed provider in Samboja	Seed provider in Samboja

Observations on land 1 with plants aged 4 months were land, the condition of the land was clean from weeds. On land 2, 3 and 4 there are weeds whose canopies connect pineapple plants to one another.

Disease Intensity

The results of observations on plants aged 4 months on land 1, found 12,174 pineapple plants with symptoms of PMWaV disease. In pineapple plants that were 8 months old in field 2, 13,845 symptomatic plants were found. In plants aged 12 months in field 3, 13,430 plants were found with symptoms. In 16 month old plants on land 4, 5,840 pineapple plants were found with symptoms. Plants with PMWaV symptoms can be seen in Figure 1. The intensity of the disease can be seen in Table 2 below.

Figure 1. Pineapple Plants with PMWaV Symptoms.

Table 2. Average Intensity of PMWaV Wilt Disease in Pineapples

Plant age	Disease intensity (%)	Attack Rate
4 months (land1)	81.16	Heavy
8 months (land 2)	92.30	Heavy
12 months (land 3)	79.00	Heavy
16 months (land 4)	73.00	Heavy

PMWaV is a member of the Ampelovirus genus in the Closteroviridae family. Wilt disease in pineapple plants (PMWaV) was first reported in Hawaii in 1910. This disease is a limiting factor that contributes to decreasing crop yields. There are two groups of diseases observed, namely rapid wilting symptoms and slow wilting symptoms. In the 1930s, this disease became a big problem in the pineapple industry until the country switched to the Smooth Cayenne variety. It turns out that this variety is not resistant to PMWaV and to date no variety has been found that is resistant to PMWaV (Dey *et al.*, 2018). This disease continues to spread to Ecuador, West Africa, Reunion Island, Mauritius and Cuba. Pineapple plants in Indonesia are not spared from PMWaP attacks. Several research results in Indonesia regarding MWaV disease and its vectors. PMWaV-1 Blitar isolates are similar to isolates from Taiwan, Mexico and Ghana, while PMWaV-2 Blitar are similar to isolates from Taiwan, Mauritius, Cuba and Ghana (Valentino *et al* .,2013). Hutahayan et.al (2021), reported that PMWaV disease in the North Tapanuli area ranged from 15% to 21.6% .

Judging from the average intensity of attacks by PMWaV wilt disease on pineapple plants in Samboja, the attack level category is heavy, namely 81.33%. The high intensity of attacks is due to farmers not knowing about the wilt disease, what causes it, how it spreads and how to control it. Only a reduction in fruit weight in the second harvest was observed by farmers. The severity of the attack on pineapple plants at the ages of 4 and 8 months can cause fruit formation to fail. In plants that are 16 months old, the plants are still able to produce but the fruit produced has decreased in weight. Pineapple farmers in Samboja District overcome plant conditions by applying chemical fertilization with TSP, ZA and Urea to stimulate the growth of pineapple plants so that they continue to bear fruit even though they are attacked by PMWaV disease. The fertilization carried out is not focused on controlling disease in plants but only to stimulate the growth and resilience of pineapple plants. The control carried out by pineapple farmers is not optimal because it does not prevent the spread of disease on the land so that losses will continue to be experienced by pineapple farmers from year to year and could even be worse. The disadvantage experienced is that the plant can only have 2 harvests in one planting, usually it can have three harvests. The weight of the fruit produced also decreased, from a pineapple plant weighing 2.5 kg to just 1.3 kg per pineapple fruit.

Disease Vectors in Land

In pineapple plantations that were attacked by PMWaV disease, mealybugs were found that were in symbiosis with pineapple plants. Mealybugs are insects that act as vectors for the disease PMWaV (pineapple mealybug wilt-associated virus) in pineapple plants. Colonies of mealybugs live on the roots or midribs and leaves of pineapple plants (Figure 1). The identification results show that the species of mealybug found in Samboja is *Dysmicoccus brevipes*. This mealybug has a slightly oval, waxy body shape and a thin pink color on its body. This is in accordance with what was stated by Hutahayan *et al.*, (2021), where the body shape is slightly oval, there are several transparent holes on the femur and tibia, the antenna consists of 8 segments and 2 pores around the eyes. The ostiol functions well, there are circulus and 17 pairs of cerarii. There is a cerarii with 3 - 4 setae on the posterior abdomen, head segments and prothorax. There is a pair of anal lobes and 2 large ribs in both (Hutahayan *et al.*, 2021). These mealybugs on pineapple belong to the Pseudococcidae family, genus Dysmicoccus, species *D.brevipes* [12].

Apart from mealybugs, ants were also found in pineapple plantations in Samboja. The spread of mealybugs is correlated with the presence of ants. Mealybugs have a mutualistic symbiotic relationship with ants, mealybugs secrete honeydew which is a food source for ants and ants protect mealybugs from attacks by their natural enemies. Ants help the spread of mealybugs and indirectly ants also help the spread of PMWaV disease more quickly (Janh & Beardsley 2000).

(a) (b)

Figure 2. Mealybug *Dysmicoccus brevipes* on the roots (a) and on pineapple leaves (b).

Controlling PMWaV disease in pineapple plants is difficult if the disease has reached a high level of severity because PMWaV wilt disease is a systemic disease. On land 4 with a plant age of 16 months and the intensity of the attack reaching 73%, no control was carried out but harvesting was carried out for planting. Appropriate control measures are really needed so that the spread of PMWaV disease can be eliminated on pineapple farming land in Samboja District.

Prevention of disease attacks can be done through proper management of planting systems. Management can be carried out by paying attention to four factors, namely clean and virus-free planting material, hot water treatment of seeds before planting (Sutrwawati *et al* 2010), controlling weeds on the land, controlling insect vectors, namely mealybugs and adjusting plant spacing. Monitoring of plant seeds is carried out by implementing plant quarantine on plant seeds that will be planted on the land. Control that can be carried out to prevent vector insects is by using natural enemies of mealybugs, namely using predators and parasitoids. Predators of mealybugs are ladybugs of the *Criptolaemus* sp. and wasps of the Encyrtidae species (Mau & Kessing 2007).

Results of Identification of Secondary Pathogenic Fungi in Pineapples

Pineapple plants that are attacked by PMWaV disease cause the plants to become more susceptible to secondary infections from secondary pathogens and the plants experience accelerated damage more quickly. Plants with symptoms other than reddening are isolated and identified as pathogens. From the identification results, pathogenic fungi were found such as *Fusarium* sp.1, *Fusarium* sp2, and *Culvularia* sp.

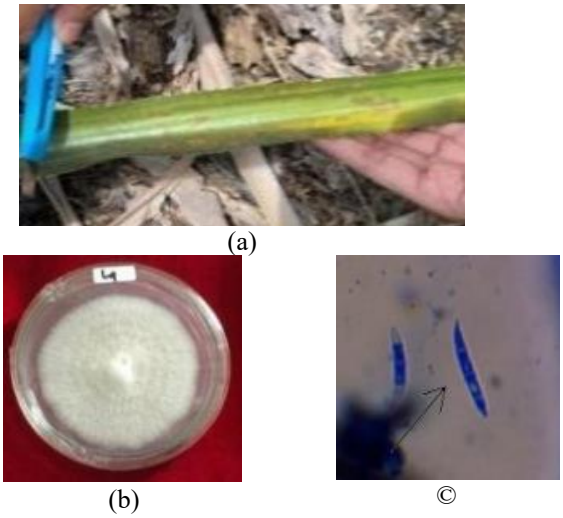
a. *Fusarium* sp1 and *Fusarium* sp2.

Fusarium sp1 fungus attacks on pineapple plants are characterized by yellowing leaves. The *Fusarium* sp1 fungus is a pathogenic fungus with a wide host distribution. The Fusarium sp fungus generally attacks the transport tissue in plants and causes wilting because it blocks the flow of water and nutrients in plants (De Cal et al 2000). Gustina (2016) has also found *Fusarium* sp1 in Queen pineapple varieties in Central Lampung. The results of isolating symptomatic leaves show white colonies with irregular edges, the fungal mycelium looks like cotton. Based on the results of microscopic observations, fungal conidia are cylindrical in shape, curved like a boat (Watanabe 2002). Symptoms of yellowing leaves, fungal colonies and microscopic fungi can be seen in Figure 3

(a) (b) (c)

Figure 3. (a) Leaves with Red Mixed with Yellow Symptoms, (b) *Fusarium* sp1 Colonies. In PDA (c) Microconidia of *Fusarium* sp1.

Yellow symptoms are also found on pineapple stalks, and will progress to the pineapple fruit, causing post-harvest fruit rot. Some Fusarium fungi can attack pineapple leaves and fruit (Martoredjo 2015). The isolation results from pineapple stalks found *Fusarium* sp2. You can see the fungal colony is bone white and the media is yellowish specifically at the center point of the colony, the surface of the colony is flatter than *Fusarium* sp 1. The hyphae are insulated, the conidia are crescent shaped. Symptoms on fruit stalks, fungal colonies and microscopic Fusarium can be seen in Figure 4.

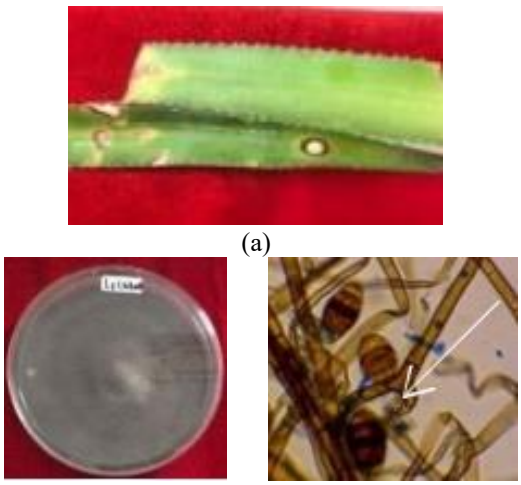


(a) (b) ©

Figure 4. Fruit Stalks with light yellow Symptoms, (b) *Fusarium* sp. Colonies on PDA, (c) Micro and Macroconidia *Fusarium* sp..

***b. Curvularia* sp.** *Curvularia sp* has been known to cause leaf spots since 2014 in Brazil, and is also reported to cause pineapple fruit rot. *Culvularia* sp was identified attacking namans plants aged 5-7, 8-11 and 12 months after planting with disease severity levels of 8.4%, 8.8% and 7.6% respectively (Gustina *et al* 2016). This fungus is actually a weak parasite and develops in the drying flower parts of the fruit ovary. The fungus enters the ovary through the honey vessels which no longer close after the fruit falls. Once the fruit is ripe, fungus develops inside the fruit (Andriani *et al.,* 2019, Ferreira *et al.,* 2014). Culvularia is also found in pineapple fruit with maturity of 25% and 75-100%. This fungus is included in the post-harvest fungus of pineapple plants, whereas previously it was only found in pineapple plantations (Butarbutar *et al.,* 2017).

In Figure 5a are the symptoms of pineapple leaves which are white in the middle and dark at the edges. Colonies of *Curvularia* sp. the resulting isolation is dark grayish black which resembles velvet (Figure 5b). Microscopically, the *Curvularia* sp fungus has dark, single or group-shaped conidia, 3 to 5 septa and hyphae that are insulated and branched at a 45 degree angle with the condia being in the form of insulated cylinders (Figure 5c). The Curvularia sp fungus is known as a pathogenic fungus that has a wide host range. The existence of the *Curvularia* sp fungus needs to receive more attention from various parties because this fungus can cause disease in plants which results in reducing production and the economic value of the affected plants (Krizsan *et al.,* 2015).



(a) (b) (c)

Figure 5. (a) Leaves with Spotting Symptoms (b) *Curvularia* sp. Colonies, (c) *Curvularia* sp. Conidia.

5. CONCLUSION

From the research results it can be concluded as follows:

1. The age of pineapple plants in Samboja District has no effect on the spread of PMWaV wilt disease on pineapple plants. On land 2, the plant age was 7 months, the highest disease intensity was 92.3% and on land 4, the plant age was 16 months, the lowest intensity of attack was 73%.
2. Secondary pathogenic fungi identified to attack pineapple plants are Fusarium sp1, Fusarium sp2 and *Curvularia* sp.

ACKNOWLEDGMENT

We would like to express our deep gratitude to the late Fachria Jumati who participated in collecting data in the early stages of this research. May Allah rewards her with goodness. We also thank all our informants who were willing to provide crucial information in the process of data collection for this research.

REFERENCES

Kementrian Pertanian. 2015. *Basis Data Ekspor-Impor Komoditi Pertanian*. Kementrian Pertanian Republik Indonesia.

Mariati, R., Yulianto, E. H., and Andraini, R. 2023. Strategi pengembangan tanaman nanas (*Ananas comosus*) di Kelurahan Bukit Merdeka Kecamatan Samboja Kabupaten

Kutai Kartanegara. *Zira'ah Majalah Ilmu Pertanian.* 48(2):298–307.

Cahyono, B. 2012. *Buku Terlengkap Budidaya Nenas secara Komersil*. Pustaka Mina. Jakarta.

Collins, J. L. 1961. *The Pineapple: Botany, Cultivation and Utilization.* Leonard Hill Interscience Publ. Inc. London.

Sutrawati, M., Suastika, G., and Sobir, S. 2010. Eliminasi Pineapple Mealybug Wilt-Associated-Virus (PMWaV) dari tanaman nanas dengan Hot Water Treatment. *Jurnal Ilmu-Ilmu Pertanian Indonesia.* 12(1):19–25.

Sether, D. M., Karasev A.V., Okumura, C.,Arakawa, C., Zee, F., Kislan, M. M., Busto, J. L., and Hu, J. S. 2001. Differentiation, distribution, and elimination of two different pineapple mealybug wilt-associated viruses found in pineapple. *Plant Disease.* 85(8):856–864.

Juarsa, A.K. 2005. *Pola Penyebaran Penyakit Layu dan Kutu Putih pada Perkebunan Nanas* (*Anans comosus* (Linn.) Merr.) *PT. Great Giant Pineapple Coy Lampung*. Skripsi. IPB University. Bogor.

Sipes, B. S., Sether, D. M., and Hu, J. S. 2002. Interactions between Rotylenchus reniformis and Pineapple mealybug wilt associated virus-1 in pineapple. *Plant Disease*. 86(9):933–938.

Dey, K. K., Green, J. C., Melzer, M., Borth, W., and Hu, J. S. 2018. Mealybug wilt of pineapple and associated viruses. *Journal Horticulture*. 4(4):52.

Valentino, H., Damayanti, T. A., Nurulita, S., Kurniawati, F., Sartiami, D., Harahap, I. S., Mubin, N., Basuki, M., Ratdiana, and Aziz, R. 2013. Detection and identification of viruses associated to mealybug wilt pineapple in Blitar, East Java, Indonesia. *IOP Conference Series: Earth and Environmental Science*. 1208(1):12013.

Hutahayan, A. J., Tantawi, A. R., and Tobing, M. C. 2021. Pineapple mealybug wilt-associated virus (PMWaV) on Sipahutar pineapple, in North Tapanuli, Indonesia. *IOP Conference Series: Earth and Environmental Science*. 782(4):42062.

Williams, D. J., and Watson, G. W. 1988 *Scale insects of the tropical South Pacific region. Part 2. Mealybugs (Pseudococcidae).* Wellingford CAB International. London.

Jahn, G. C., and Beardsley, J. W. 2000. Interactions of ants (Hymenoptera: Formicidae) and mealybugs (Homoptera: Pseudococcidae) on pineapple. 34:161–165.

Mau, R. F. L., and Kessing, J. L. M. 2007. Dysmicoccus brevipes (*Cockerell). The Crop Master Program*. *Extension Entomology and UH-CTAHR Integrated Pest Management Program*.

De Cal, A., Garcia-Lepe, R., and Melgarejo, P. 2000. Induced resistance by *Penicillium oxalicum* against *Fusarium oxysporum* f. sp. lycopersici: Histological studies of infected and induced tomato stems. *Phytopathology*. 90(3):260–268.

Gustina, M., Ratih, S., Nurdin, M., and Suharjo, R. 2016. Inventarisasi patogen di pertanaman nanas (*Ananas comosus* L.) varietas queen di Desa Astomulyo, Kecamatan Punggur Kabupaten Lampung Tengah. *Jurnal Agrotek* Tropika. 4(3):205–210.

Watanabe, T. 2002. *Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species*. CRC press.

Martoredjo, T. 2015. *Ilmu penyakit pascapanen*. Bumi Aksara. Jakarta.

Andriani, S., Aini, F., and Ihsan, M. 2019. Isolasi dan identifikasi jamur patogen pada tanaman nanas (*Ananas comosus* (L). Merr) Varietas Tangkit. *Journal Bio-site*. 5(1):12–20.

Ferreira, A. P. S., Pinho, D. B., Machado, A. R., and Pereira, O. L. 2014. First report of curvularia eragrostidis causing postharvest rot on pineapple in Brazil. *Plant Disease.* 98(9):1277.

Butarbutar, R., Dirmawati, R. S., Nurdin, M., and Suharjo, R. 2017. Identifikasi jamur pada buah nanas (*Ananas comosus* L.) Kultivar MD2 pada berbagai tingkat kemasakan. *Journal Agrotek Tropika.* 7(3):397-404.

Krizsán, K., Ppapp, T., Manikandan, Shobana, Chandrasekaran, Vagvolgyi and Kredics, L. 2015. Clinical importance of the genus Curvularia. *Medical Mycology: Current Trends and Future Prospects. CRC Press, Boca Raton FL.*