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Effectiveness of Leaf Extracts : Soursop, Kaffir Lime, Piper Betle and A Mixture of The Three to Control Downy Mildew of Maize Plants

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Abstract

This research aims to determine the effectiveness of soursop leaf extract, kaffir lime leaf extract, piper betle leaf extract and a combination of the three as a botanical fungicide to control maize downy mildew. Maize plants in this study were planted in soil media in polybags using a Complete Randomized Design 1 factor with 9 levels of treatment : H0 (no fungicide), H1 (soursop leaf extract 100g/l distilled water), H2 (kaffir lime leaf extract 100g/l distilled water), H3 (piper betle leaf extract 100g/l distilled water), H4 extract of (soursop leaf 50g+kaffir lime leaf 50g)/l distilled water), H5 extract of (soursop leaf 50g+piper betle leaf 50g)/l distilled water), H6 extract of (kaffir lime leaf 50g+piper betle leaf 50g)/l distilled water), H7 extract of (soursop leaf 33.3g+kaffir lime leaf 33.3g+piper betle leaf 33.3g)/l distilled water) and H8 (inorganic fungicide Cupric Oxide). The results of the research showed that the extract application of soursop, kaffir lime, piper betle and a mixture of the three resulted in the intensity of maize downy mildew ranging from 18.50%-22.91%, equivalent to the application of cupric oxide of 20.25%, while without fungicide treatment the intensity of downy mildew was 48.16%. Therefore, these three botanical materials have the potential to be used as botanical fungicides to control downy mildew disease in maize plants. The three botanical extracts support better growth and yields of maize plants. A mixture of (soursop leaf 33.3g+kaffir lime leaf 33.3g+piper betle leaf 33.3g)/l distilled water (H7) gave the heaviest seed weight 195.55g/plant.

Keywords : leaf extracts, downy mildew control, maize

Introduction

Maize is an important cereal commodity besides rice and wheat which is needed by the world's population as a staple food. This commodity produces 42 percent of calories as the main source of energy and 37 percent of protein intake, micronutrients and various non-nutritive bioactive food components (Olaf et al., 2022; Poole et al., 2021). Apart from being a source of carbohydrates, maize also contains fiber, vitamins, minerals and natural antioxidants which are useful for maintaining health and preventing chronic diseases in humans such as : cancer, diabetes, obesity, cardiovascular, bladder and stomach disorders (Tapas and Chikkappa, 2022). Apart from human consumption, maize is also widely used as animal feed and biofuel (Shraddha and Vijay, 2021).

This cereal crop is widely planted in tropical and subtropical regions of the world in large quantities. In Indonesia, in 2023 the harvest area will be 2.93 million ha, dry-shelled maize production will reach 14.77 million tons with a water content of 14% (Official Statistics News BPS-Statistics Indonesia No. 21/03/Th. XXVII, 2024). Therefore, strategies for developing maize plants that are adaptive to the biotic and abiotic environment continue to be pursued in order to obtain increased production and food security (Ishfaq et al., 2020). According to Sherry et al. (2020), as an important and nutritious staple food, maize has cultural, economic, environmental and nutritional impacts to support the health of the world's population.

Several countries continue to strive to increase maize production, including Indonesia with a free hybrid maize seed program through the Special Efforts Program and intensification and extensification programs (Official Statistics News BPS-Statistics Indonesia No. 21/03/Th. XXVII, 2024). In China, this is done by increasing plant density. Increasing maize density from 60,000 to 75,000 plants per ha with the same Nitrogen input could increase maize yields by 5.59% across China (Peng et al., 2020). In Sub-Saharan Africa, increasing maize production is carried out by developing small farmers, using technology and preserving plant diversity (Romy, 2020).

One of the most destructive diseases in maize plants is downy mildew caused by the fungus *Perenosclerospora sp.* This pathogen also attacks millet, sorghum and sugar cane plants in tropical climates throughout the world (J.A. Crouch et al., 2022). In humid subtropical and tropical areas in Asia, especially in monoculture maize plantations, downy mildew disease can develop very seriously and cause a loss of 80%-100% of maize yields (Muis et al., 2023; Ummu et al., 2015). *Perenosclerospora sp*. causes downy mildew is able to survive in seeds, sometimes forming resting spores with the ability to survive for more

than 1 year. The pathogen is also able to spread quickly and aggressively so that this disease has the potential to become a serious threat to maize production (Muis et al., 2023; Ummu et al., 2015). The fungus is obligate, sporulation occurs mostly at night temperatures of 21–23°C with high humidity, oospores can survive in the soil and can spread through infected seeds (Hyo et al., 2020).

According to Amran et al. (2016), in Indonesia the pathogen has spread almost throughout the island. In an area more than one species can be found, on the other hand, one species can be found in several areas where downy mildew is endemic. *P. philippinensis* is only found on Sulawesi Island, while *P. maydis* and *P. sorghi* are found on all islands in Indonesia. Farmers generally control plant diseases with anorganic fungicides, even during the 20th century chemical pesticides played a very important role in increasing maize yields in the United States and wheat in England (Vinay et al., 2022). The use of inorganic pesticides has many negative impacts on soil and water systems, plant metabolic processes, human health through skin and breathing as well as resistance to plant pest organisms (Koutroubas and S.D., 2016; Vinay et al., 2022).

Maize downy mildew control is often done with metalaxyl fungicide, but this chemical tends to make the fungus resistant and increases the price of maize seeds (Zerka et al., 2013). According to Jainuddin et al. (2018) treating maize seeds using metalaxyl is not effective for controlling maize downy mildew. It was reported that repeated use of benzimidazoles and strobilurins causing to increased tolerance of powdery mildew fungi (Munekazu et al., 2023). Due to the many negative effects of using inorganic fungicides, alternative methods of controlling maize downy mildew are needed that are more ecofriendly.

Botanical fungicides are an ecofriendly alternative of fungicides because they are easily degraded, besides that these botanical materials are often not utilized optimally and are often simply thrown away. Botanical materials contain secondary metabolites where these secondary metabolites have a function for body defense and several ecological functions of the plant so that they can be used as pesticides (Seyed et al., 2020). According to (Joko et al., 2021), a combination of piper betle extract fungicide with the biological agent *T. asperellum* can reduce the incidence of downy mildew, extend the incubation period, and increase the dry weight of maize shoots. This research aims to determine the potential of kaffir lime, piper betle, soursop leaf extracts and a combination of the three as botanical fungicides to control maize downy mildew.

Materials and Methods

The research was carried out in Karangsari Village, Kembaran District, Banyumas Regency, Central Java, Indonesia, at 90 m alt starting March 2023 until June 2023. Using Complete Randomized Design 1 factor with 9 levels of treatment, namely: H0 (no fungicide), H1 (soursop leaf extract 100g/l distilled water), H2 (kaffir lime leaf extract 100g/l distilled water), H3 (piper betle leaf extract 100g/l distilled water), H4 (soursop leaf extract 50g+kaffir lime leaf extract 50g)/l distilled water), H5 (soursop leaf extract 50g+piper betle leaf extract 50g)/l distilled water), H6 (piper betle leaf extract 50g+kaffir lime leaf extract 50g)/l distilled water), H7 (soursop leaf extract 33.3g+kaffir lime leaf extract 33.3g+piper betle leaf extract 33.3g)/l distilled water) and H8 (Cupric Oxide fungicide).

Planting of the Pertiwi-6 variety of maize is carried out in soil media placed in polybags. Each treatment was repeated 4 times with a distance of 150 cm between polybags. Spraying of botanical fungicides is carried out from the time the maize seeds grow at intervals of every 7 days up to 14 days before the maize plants are harvested.

Results and Discussion

Table 1. Intensity of Downy Mildew, Plant Height, Leaf Area of Maize Plants on Botanical Fungicide Treatment

Treatment	Intensity of Downy Mildew (%)	Plant Height (cm)	Leaf Area (cm^2)
H0	48.16 b	211.00	4060.65
H1	20.25 a	246.25	4661.08
H2	19.17 a	241.00	4845.65
H3	22.91 a	240.75	4287.40
H4	21.00 a	252.50	4669.63
H5	20.75 a	251.00	5068.08
H6	18.50 a	257.75	4807.10
H7	20.33 a	248.75	4534.60
H8	20.25 a	248.75	4575.53

Note : Numbers followed by different notations in 1 column are significantly different (DMRT 5%).

The data in Table 1 shows that botanical fungicide treatment resulted in downy mildew disease intensity ranging from : 18.50%-22.91%, which is lower than without fungicide treatment : 48.16%. The three botanical materials showed no significant effect on the growth of plant height and leaf area. It is possible that genetic factors dominate in influencing the growth of plant height and leaf area of maize plants.

Table 2. Number of Cobs, Weight of Cobs with Husk, Weight of Cobs Without Husk, Weight of Seeds of Maize Plants under Botanical Fungicide Treatment

Treatment	NC/Plant	WCH/Plant (g)	WCWH/Plant (g)	WS/Plant (g)
H0	1	212.40 a	147.00 a	94.08 a
H1	1	298.63 b	227.45 b	165.83 b
H2	1	331.78 b	251.00 b	181.15 b
H3	1	294.68 b	219.33 b	156.28 b
H4	1	291.55 b	234.68 b	171.08 b
H5	1	339.25 b	245.88 b	174.63 b
H6	1	326.98 b	245.68 b	178.38 b
H7	1	336.43 b	261.95 b	195.55 b
H8	1	290.30 b	227.08 b	168.68 b

Note : Numbers followed by different notations in 1 column are significantly different (DMRT 5%).

NC : Number of Cobs/Plant

WCH : Weight of Cobs with Husk/Plant

WCWH : Weight of Cobs Without Husk/Plant

WS : Weight of Seeds/Plant

From the data shown in Table 2, it shows that in this study the maize variety tested produced 1 cob/plant according to the potential of the maize variety planted, namely Pertiwi-6. Treatment without fungicide application (H0) produced maize cobs that were significantly smaller and produced lighter seed weight. It can be seen that maize plants without fungicide application (H0) show lower maize cob yields compared to those treated with botanical fungicides and Cupric Oxide fungicides. Maize plants without fungicide application showed low yields : weight of cobs with husk was 212.40 g/plant, weight of cobs without husk was

147.00 g/plant and weight of seeds was 94.08 g/plant. The low yield in treatment without fungicide application was due to the high intensity of downy mildew : 48.16%.

Soursop leaf extract contains phenols, polyphenols, terpenoids, alkaloids, flavonoids, saponins, phytosterols and aromatics which have broad spectrum anti-fungal properties. It was reported that soursop extract efficiently inhibited the growth of *A. alternata* fungal mycelia on tomatoes in vitro (Rizwana et al., 2021). Soursop leaf extract also contains phytol, neophytadiene, hexadecanoic acid, and trans-caryophyllene (Deewatthanawong et al., 2019).

Kaffir lime leaf extract contains alkaloids, flavonoids, tannins, steroids, triterpenoids, essential oils and phenols (Suryani et al., 2022). According to Krishnamoorthy et al. (2023), *Citrus hystrix* leaf extract mediated by ZnO NPs provides anti-fungal activity against *Candida albicans* and *Aspergillus niger*. ZnO NPs also have good antioxidant activity. Kaffir lime leaf extract has the potential to be a natural antimicrobial against the fungus *P.oryzae* which causes blast disease in rice. *C. hystrix* extract concentrations : 10, 20, 30 mg/ml have an effect on the inhibitory zone diameter of *P.oryzae* colonies, respectively : 6.33 mm, 6.67 mm, 7.33 mm (M.N.A. Uda et al., 2018).

Piper betle leaf extract contains tannins, flavonoids, the aromatic compounds hydroxycavicol, cavicol, and betlepenol which are able to control the growth of pathogenic fungi by inhibiting cell wall permeability, causing proteins to leave the cells and the fungal cells to gradually die (Koul et al., 2008). According to Aoki et al. (2019), the methanol extract of *P. betle* leaves contains 4-allylpyrocatechol, eugenol, α-pinene, and β-pinene which show an inhibitory effect on the fungus that causes grape downy mildew disease. *P. betle* leaf botanical fungicide is also effective in controlling *Phakopsora pachyrhizi* leaf rust disease in soybean plants. The control treatment (without fungicide) showed a percentage of soybean rust attack of 6.9%, while the *P. betle* extract treatment with a concentration of 0.75% resulted in a percentage of soybean rust attack of 2.1% (Simbolon et al., 2022).

Although the three plant-based fungicides contain different secondary metabolites, they provide relatively similar control effects on maize downy mildew. The effectiveness of the three botanical materials ingredients is also the same as the inorganic fungicide Cupri Oxide. The content of flavonoid compounds in plant extracts functions to deter microorganisms and insects (Muhammed and Manan, 2015), likewise alkaloids function as poisons for microorganisms and insects (Wink, 2007). Tannin compounds are antimicrobial (Chung et al., 2016; Tshabalala et al., 2019) and also function as antioxidants in plants to prevent or inhibit plant cell damage (Muhammed and Manan, 2015). Saponins can improve

the immune system through adjuvant activity and function as anti-parasitic, anti-bacterial and anti-viral compounds (G et al., 2007).

Maize plants that were not applied with fungicides showed a higher intensity of downy mildew disease (48.16%) resulting in low yields. A high intensity of downy mildew is correlated with less healthy leaf surface and lower chlorophyll levels, causing the photosynthesis process to run less than optimally as well as smaller cob yields and fewer seeds. According to Mierziak et al. (2014), flavonoid compounds have a functional role in acclimatization to extreme high and low temperatures and also play a role in defending plants against drought stress. Flavonoids can neutralize several cases : high doses of ultraviolet-B light, excess metal ions and imbalances in photosynthetic oxidation-reduction processes as well as influencing signals for the formation of the phytohormones auxin and abscisic acid (Brunetti et al., 2018).

Alkaloids function as growth regulators and as storage compounds that are able to supply nitrogen and other elements needed by plants (Wink, 2007). Alkaloid compounds act as nitrogen storage reservoirs where these compounds will be released from specific gland organelles and exported to target tissues that experience stress signals from the biotic and abiotic environment (Sweta et al., 2021). Terpenoid compounds act as antioxidants for plants to support extensive plant growth (Babychan and Jk, 2017). Terpenoid compounds react directly with oxidants intracellularly and on the leaf surface to protect plants from abiotic stress, for example thermal stress which can cause : membrane destabilization of the thylakoid membrane, membrane fragmentation and disintegration of protein complexes (Delbert et al., 2020).

According to (Mohandas and Kumaraswamy, 2018), high levels of terpenoids correlate with high antioxidant properties in plants. According to Atanu et al. (2020), tannin compounds act as plant defense molecules. This compound provides a barrier against the invasion of phytopathogens in plants so that it can prevent disease in plants. Tannin compounds not only function to support plant resistance to pathogens, but are also antioxidants that protect plant structures from negative effects in the form of oxidative stress, light stress and physical injury (José et al., 2022).

Of the three botanical ingredients tested, namely kaffir lime leaf extract, piper betle leaf, soursop leaf and a mixture of the three, they contain different types of secondary metabolites and possibly also levels. The three botanical ingredients showed a fungicidal effect on the fungus that causes maize downy mildew. These secondary metabolite compounds also have positive effects, including neutralizing the effects of abiotic stress, such

as extreme hot and cold temperature fluctuations and drought conditions, so that they can support more optimal plant growth and development and produce better maize cobs.

Conclusion

Extract application of soursop leaf, kaffir lime leaf, piper betle leaf and a mixture of the three can significantly reduce the intensity of maize downy mildew compared to without fungicide treatment. Therefore, these three organic materials have the potential to be used as botanical fungicides to control downy mildew disease in maize plants.

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