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Bandotan (*Ageratum conyzoides* L.) as Bio-Fungicide for Controlling *Fusarium oxysporum* in Chili (*Capsicum annuum* L.)

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Abstract. *Fusarium oxysporum L. is a fungus responsible for causing plant wilt disease in various horticultural crops. Meanwhile, Bandotan (Ageratum conyzoides L.) is commonly known as a weed that contains secondary metabolites with antifungal activity. Therefore, this study aimed to determine the effect of Bandotan stem and leaf extract on the growth of Fusarium oxysporum in chili (Capsicum annuum L.) infected during the germination phase. Evaluation of stem and leaf extract was carried out separately using a one-factor Complete Randomized Design (CRD). A total of four experimental units were used consisting of C+ (sprouts not infected with Fusarium sp. and not given stem or leaf of Bandotan extract), C- (sprouts infected with Fusarium sp. but not given stem or leaf of Bandotan extract), T1 (sprouts soaked with stem or leaf of Bandotan extract and infected with Fusarium sp.), and T2 (sprouts infected with Fusarium sp., and given stem or leaf of Bandotan extract). Each experimental unit was repeated five times, and parameters observed included disease severity, as well as dry and wet weight. The results showed that based on ANOVA test at the 5% significance level, the administration of 60% dry Bandotan stem extract to chili sprouts before Fusarium sp. infection significantly reduced disease severity. However, when the treatment was carried out after sprouts were infected, disease severity was not reduced. The administration of Bandotan leaf extract to sprouts both before and after being infected with Fusarium sp. reduced disease severity but a decrease in dry weight was also observed.*

Keywords: *Ageratum conyzoides L., Fusarium sp., bio-fungicide, Capsicum annuum L.*

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INTRODUCTION

Fusarium oxysporum is a crucial pathogen affecting the growth and yield of horticulture crops. This soil-borne disease causes fusarium wilt (Sutarini et al., 2015) infecting chili (*Capsicum annuum* L.) plant from germination to maturity (Kaisah, 2021). Infection usually occurs through wounds and then spread to all parts of the plant. The initial symptom is the yellowing of the lower leaf which subsequently wilt as the plant matures (Ghufron et al., 2017; Araz, 2014). This phenomenon occurs only on one side of the plant, leading to drooping, browning, and eventually drying off. Based on a previous report, diseased plant stem often show brown vascular bundles when split (Gufron et al., 2017). Further infection spreads to stem and causes the plant to wilt, resulting in the production of smaller fruits which may fall off (Araz et al., 2014). According to Corteva (2020), wilting symptoms due to *Fusarium oxysporum* are particularly evident during the day, with plant returning to being fresh in the evening and morning (Corteva, 2020).

Farmers generally use synthetic fungicides to control fusarium wilt that infects plant. Fungicides are produced using chemicals and continuous use at excessive doses can lead to environmental pollution or cause poisoning (Febia et al., 2020). Therefore, it is necessary to control pathogenic fungi that are more environmentally friendly by using natural fungicides derived from plant (Umboh, 2019).

Bandotan (*Ageratum conyzoides* L.) is a weed plant known to contain secondary metabolites including precedence I and II, saponins, flavonoids, polyphenols, and essential oils (Febia et al., 2020). According to Atisha and Mita (2018), Bandotan has benefits for wound healing due to the pharmacological effects as an antibacterial. The pharmacological effects are attributed to the active compounds including saponins, alkaloids, terpenoids, and phenols. These secondary metabolites commonly found in the leaf, stem, and root, have antimicrobial and antifungal, anti-edema, anti-inflammatory, and antioxidant properties (Cahyani & Mita, 2018). Shintya et al. (2014) proved that Badotan leaf extract was effective in suppressing the growth of fungus Colletotrichum capsici known to cause anthracnose in various fruits.

Studies on the active compounds of Bandotan have generally been aimed at determining the antibacterial activity against microbes that cause human diseases. However, investigations on the antibacterial and antifungal activities against microbes that attack crops are few. This study aimed to evaluate the potential antifungal activity of Bandotan leaf and stem extract against pathogenic fungi that attack different horticultural crops. The results are expected to support the achievement of the twelfth SDG's goal of Responsible Consumption and Production, specifically in the field of food production. The proper use of bandotan extract to treat fungal pathogens of horticulture crops not only avoids environmental pollution due to the continuous and excessive use of synthetic fungicides but also ensures that crop yields are free from chemical contaminants.

MATERIALS AND METHODS

This study was conducted from May to July 2022 at the Microbiology and Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung. Bandotan plant was collected from areas around Bandar Lampung, while chili seeds were obtained from an agricultural shop. Additionally, *Fusarium* sp. isolates were acquired from the collection at the Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung.

Study Design

This study was conducted separately between leaf and stem extract using a Completely Randomized Design (CRD) with one treatment factor, namely Bandotan stem or leaf extract consisting of four treatment levels as follows:

C+ = chili sprouts not infected with *Fusarium* sp. and not given stem or leaf of Bandotan extract. C- = chili sprouts infected with *Fusarium* sp. without being given stem or leaf of Bandotan extract. T1 = chili sprouts soaked in the stem or leaf of Bandotan extract and then infected with *Fusarium* sp. 7 days after planting (DAP). T2 = chili sprouts are soaked with *Fusarium* sp. and then given stem or leaf of Bandotan extract 7 days after planting (DAP). All treatments were repeated five times and the parameters measured included plant height, leaf area, wet and dry weight, as well as disease severity measured at 33 DAP.

Chilli seed germination was carried out in a petri dish that had been coated with wet germination paper according to the treatment. Sprouts were then planted in polybags containing planting media consisting of soil and humus in a ratio of 2:1 according to the treatment. All polybags were placed in the greenhouse of the Botany Laboratory of the Biology Department.

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Study Implementation

Preparation of Bandotan Stem Extract

This study was conducted by preparing wet and dry extract of Bandotan stem and leaf separately. The preparation of Bandotan dry extract was carried out by following the method used by Anggraini et al. (2022) with slight modifications. Clean fresh stem or leaf was dried in the sun for 7 days to remove the water content and then pulverized into powder. A total of 500 g powder was soaked in 2 L methanol for 24 hours, then the macerate was filtered using a glass funnel and a filter paper followed by concentration with a rotary evaporator until a thick extract was obtained. Stem or leaf used for treatment was 60% dry extract (weight/volume), determined based on the results of the inhibition test against the growth of *Fusarium* sp. in vitro.

Preparation of *Fusarium* sp. Isolates

Fusarium oxysporum isolates were rejuvenated on PDA in a petri dish and then incubated at room temperature for approximately 2 weeks until white fungal monospores were obtained (Arsih, 2015). The concentration of Fusarium spore suspension used in this study was 107 spores per mL.

Application of Bandotan Extract and *Fusarium* sp. Infection on Chili Plant

Chili sprouts used in this study had a radicle length of about 2-3 mm. In C and T2 treatments, sprouts were soaked in a suspension of *Fusarium* sp. with a concentration of 107 spores mL-1 for 1 hour, then planted in polybags containing sterile media. In T1 treatment, chili sprouts were soaked in 60% dried Bandotan stem or leaf extract for 1 hour, followed by planting in polybags containing sterile media. The composition of the sterile media was 3 soil:1 compost.

Infection of *Fusarium* sp. (T1 treatment) and application of dried Bandotan stem or leaf extract (T2 treatment) to the test plant in polybags was carried out 7 days after planting (DAP). This was conducted by inserting a suspension of *Fusarium* sp. (107 mL-1) or 60% dried Bandotan extract in holes 2 cm away from the test plant with a depth of 2 - 3 cm (Figure 1).

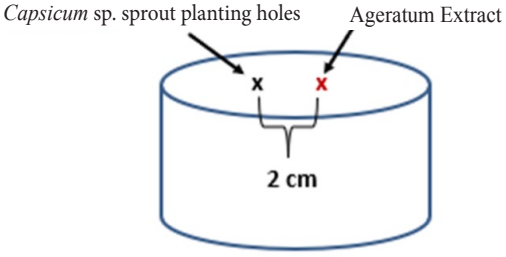

Figure 1. 2-3 cm deep holes about 2 cm away from chili plant for *Fusarium* sp. infection as well as dried Bandotan stem or leaf extract.

Plant Height

Total Plant height was measured at 14 DAP, specifically, chili was harvested and then cleaned from the attached soil. Measurement was carried out from the tip of the root to the shoot (Novitasari, 2019) using thread. The length obtained was then measured using a ruler.

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Plant Wet and Dry Weight

The wet and dry weights of chili plant were measured at 33 DAP by removing from the polybags followed by cleaning and weight measurement while still fresh using a digital scale (Dumayanti, 2021). Chili plant was dried in an oven at 80°C for 24 hours and weighed using a digital scale (Dumayanti, 2021).

Leaf Area Measurement

Measurement of leaf area was carried out using the method by Dumayanti (2021). All leaf on the observed plant were picked and made replicas on HVS paper. The replicas obtained were cut out and weighed, then as a standard, the same piece of HVS paper measuring 10 cm x 10 cm was made and weighed. L area was calculated using the formula:

$$LD = \frac{Weight\ of\ leaf\ replicas}{paperweight\ 10 \times 10} \times 100\ cm^2$$

Description:

LD = leaf area (Dumayanti, 2021)

Disease Severity

Disease severity was calculated using the Ruliyanti method (2020) with the formula:

$$SD = \sum \frac{n \times v}{N \times V} \times 100\%$$

Based on above formula, *SD* is the severity of disease, *n* is the number of infected plant in each category, *N* is the number of plant observed, *v* is score value of each category, and *V* is the highest score value. The score for calculating disease severity used a scale of 1 - 5 (Ambar et al., 2010) whereas scale 1: healthy plant, no wilting, scale 2: 0 - 25% leaf wilting, some leaf starting to wilt, scale 3: 26 - 50% leaf wilting, almost all leaf wilting, scale 4: 51 - 75% leaf wilting, all leaf are wilting, but the stem is still fresh, and scale 5: 76-100% leaf wilting, plant death.

Data Analysis

The data obtained were analyzed for variance, and DMRT was further tested at a 5% significance level. The study of stem and leaf extract was conducted separately using the same design.

RESULTS AND DISCUSSION

According to Chabal et al. (2021), the various secondary metabolites contained in Bandotan include flavonoids, chromenes, chromones, coumarins, benzofurans, terpenoids, steroids, and alkaloids, facilitating the use as a natural fungicide, fumigant, antifungal, and antimicrobial. Antifungal bioactive compounds comprise procene II and eugenols (Chabal et al., 2021). The highest concentration of flavonoids in Bandotan is found in stem (Melisa & Muchtaridi, 2017). ANOVA test at the 5% significance level showed that the 60% dried Bandotan stem extract had a significant effect on plant growth and survival of *Fusarium oxysporum*. This was evidenced in the severity of Fusarium wilt disease (Figure 2) in chili.

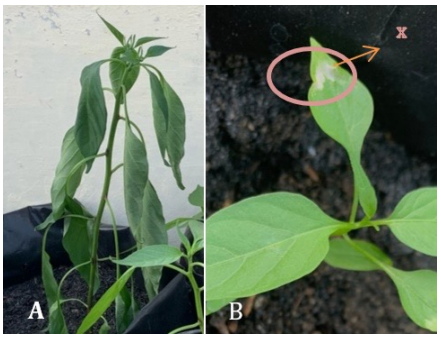

Figure 2. Symptoms of Fusarium wilt on chili plant derived from: (A) sprouts soaked with *Fusarium* sp./K- and (B) sprouts soaked with Fusarium sp. then treated with Bandotan leaf extract/T2. Whereas, x = Leaf that experience chlorosis and yellowing

The application of Bandotan dry extract reduced the severity of fusarium wilt

disease, suggesting the secondary metabolite content has bio-fungicide potential. These results were consistent with Arie et al. (2015) where 10% fresh Bandotan extract reduced severity of anthracnose disease in banana plant caused by the fungus *Colletotrichum musae*. Spraying dry Bandotan ethanol extract (2 mg/ml in 0.2% DMSO) on rice plant effectively reduced the severity of blight caused by infection with Pyricularia oryzae Cavara and Rhizoctonia solani Kühn fungi (Nguyen et al., 2021).

In vitro tests of Bandotan dry extract have been conducted against *Pyricularia oryzae* Cavara and *Rhizoctonia solani* Kühn which are pathogenic fungi of rice plant (Nguyen et al., 2021). Javed and Bashir (2012) proved that the dried flower, leaf, stem, and root extract of Bandotan at a concentration of 2% reduced the dry weight of *Fusarium solani* culture products.

Figures 3a to 3d show the comparison of plant morphology and disease severity between the results of the treatment. The dried leaf extract treatment produced higher plant height, leaf area, as well as wet and dry weight than the 60% stem extract treatment. Similarly, administering the 60% Bandotan leaf extract treatment either before (T1) or after (T2) *Fusarium* sp. infection led to a significantly lower disease severity compared to sprouts only infected with Fusarium sp. (C-). This outcome is presumably due to the higher content of secondary metabolites in leaf than in stem.

Leaf serves as an organ for photosynthesis to provide food or energy sources. Biosynthesis of secondary metabolites in plant occurs through three main pathways, namely 1) the cyclic acid, 2) the mevalonic acid and methylerythritol phosphate (MEP), as well as 3) the malonic pathway. These three secondary metabolite biosynthesis pathways use precursors which are intermediary compounds in carbon metabolism, namely the respiration-photosynthesis process (Twaij and Hasan, 2022). Therefore, apart from being a food-producing organ or energy source, namely carbohydrates, leaf also acts as a place for the biosynthesis of various secondary metabolites. Atisha and Mita (2018) showed that the secondary metabolites in Bandotan leaf were more diverse and higher compared to stem, root, and flower.

Fusarium oxysporum infects plant through wounds on the root then develops to stem and extends to the vascular tissue, resulting in decay of the transport tissue as well as obstruction of water and nutrient absorption, leading to wilting (Kristiawati et al., 2014). Based on the results, treatment C- produced plant that showed symptoms of *Fusarium* sp. attack with the highest disease severity of 17.25 and 43.75% (Figure 3e). Wilting in plant infected with Fusarium sp. is presumably attributed to the significantly low water content, making leaf cells lose turgor. According to Wang et al. (2015), a decrease in relative water content due to the inhibition of transport through the xylem causes pathological wilting of foliage.

Figure 3e showed that although the 60% Bandotan dried stem extract treatment reduced disease severity, the result was not significantly different from treatment C-. A significant reduction in disease severity was observed in T2 treatment, where the dried stem extract was given after the plant was infected with *Fusarium* sp. Bandotan is recognized as a dominant weed in various crop cultivation, specifically in plantations (Sari & Jainal, 2020) thriving in rice fields, gardens, yards, and roadside (Sultan et al., 2016). This plant releases allelopathic compounds, which are chemical exudates excreted into the environment and potentially cause

growth inhibition of other surrounding plant (Kusuma Wardani et al., 2018). According to Isda et al. (2013), the accumulation of allelochemicals in plant cells inhibits the growth of other plant by making cells become inelastic and transferring ions through cell membranes. Nasrin (2013) reported that three phenolic acids namely gallic, coumaric, and proteocatechins were allelopathic compounds in Bandotan.

Bandotan extract treatment ranging from 5% to 20% decreased plant height, wet

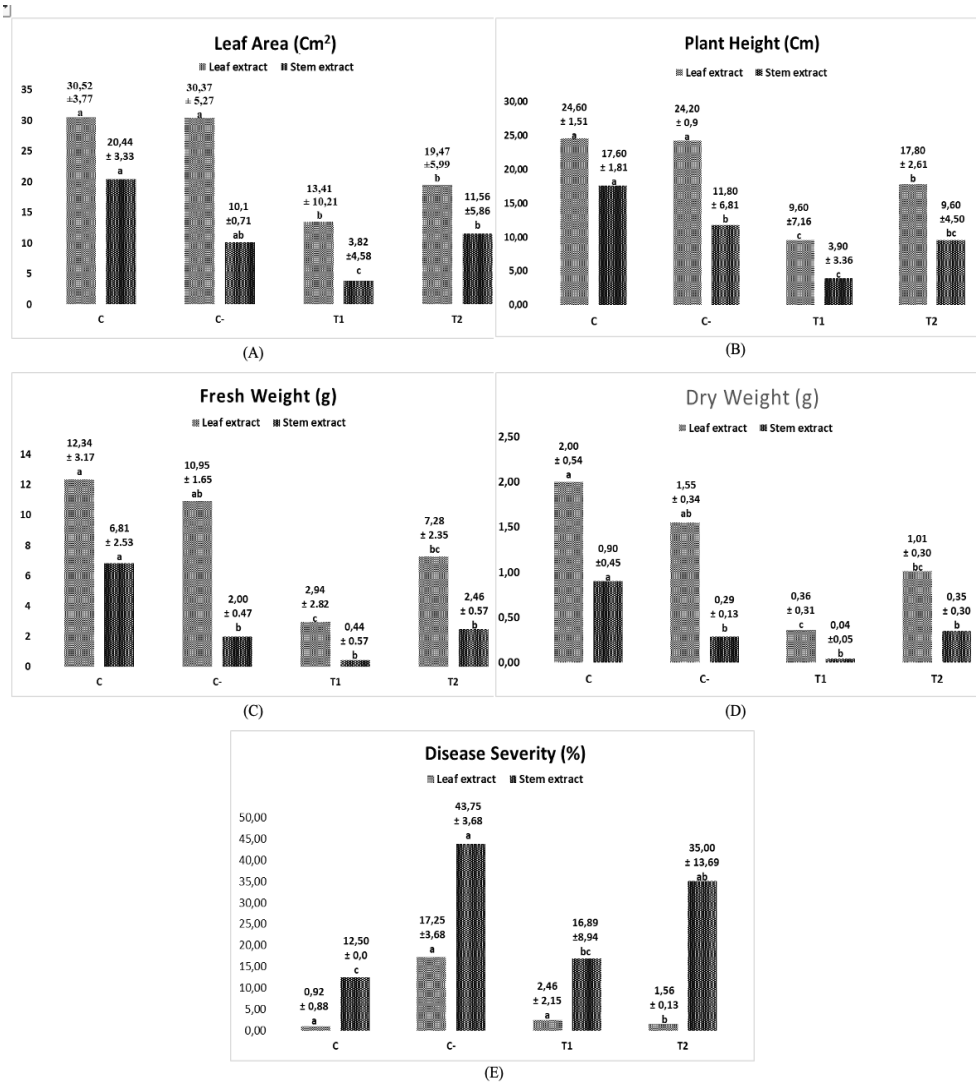

Figure 3. The effect of the method of application of dried stem or leaf extract of Bandotan Ageratum conyzoides L.) on the growth of chili plant (Capsicum annuum L.) infected with Fusarium sp. infected with Fusarium sp. Numbers on the same colored bars followed by the same letter indicate not different at the significance level = 5%. C = concentration, T = treatment

and dry weight, growth tolerance index, as well as a, b, and total chlorophyll. This result was consistent with a previous study where the largest decrease in plant height was obtained from administering the highest concentration extract did not prevent Fusarium wilt disease when the plant was subsequently infected with *Fusarium* sp. 7 DAP (T2 treatment). On the other hand, the extract significantly reduced wilt disease when given to plant previously infected with *Fusarium* sp. (T1 treatment).

The application of dry leaf extract to sprouts previously infected with Fusarium sp. (T1) and those newly infected (T2) 7 DAP, significantly reduced the severity of the disease. These results were in line with Shintya et al. (2014) where Bandotan leaf extract effectively suppressed the growth of fungus *Colletotrichum capsici* known to cause anthracnose on various fruits.

Further studies are needed to elucidate the differences in bio-fungicidal activity of leaf and stem extract. This is because as a weed plant, the allelopathic compounds in Bandotan are not limited to the two organs. Sari and Jainal reported that fresh Bandotan leaf extract (200gr leaf/200 ml water) demonstrated an allelopathic effect causing a decrease in mung bean seed germination by 53.33%. Furthermore, Negi et al. (2020) mentioned that the allelopathic activity of Bandotan root and leaf extract caused inhibition of rice germination. Both allelopathic and fungicidal activities of Bandotan extract are derived from secondary metabolic compounds. All organs of the plant are known to contain various contents with different types and concentrations (Paul et al., 2022; Athisa and Mita, 2018). The biological activity of secondary metabolites is also influenced by the extraction method used (Shintya et al. 2014).

The reduction in disease severity due to Bandotan extract treatment did not maintain dry weight content (Fig. 3d vs. 3e). This suggests that the decrease in water content due to *Fusarium* sp. infection not only caused wilting but also affected the metabolic processes, disrupting the photosynthesis process. According to Yan et al. (2018), wilting of foliage causes damage to photosystem I (PS I) and II (PS II), inhibiting the photosynthesis process. PS I is generally more drought-tolerant than PS II (Zivcak et al., 2014; Zhang et al., 2016). In plant that experience severe drought stress, damage to PS I is greater than PS II (Huang et al., 2013).

Despite the need for further investigation regarding bio-fungicidal potential of Bandotan extract, the results show a promising prospect as a source of bio-fungicide. Exploring optimal extraction methods is essential to easily obtain bio-fungicide from Bandotan, given the abundance, affordability, and potential to reduce the risk of environmental pollution associated with the continuous use of synthetic fungicides

CONCLUSION

In conclusion, dried extract of Bandotan showed bio-fungicidal activity against Fusarium sp. infecting chili plant. Bio-fungicidal activity of 60% Bandotan dried leaf extract was greater compared to stem. However, the underlying reasons for the difference are currently unknown. This underscores the need for further studies into the biological activity of various secondary metabolite compounds contained in each organ of Bandotan plant.

AUTHOR CONTRIBUTION

N. F and D.E.T designed the research, collected, and analyzed the data and R.A, L.C, Y., and M supervised all the process and R.A and L.C also wrote and layouted the manuscript.

CONFLICT OF INTEREST

There is no conflict of interest in this research.

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