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Trichoderma Harzianum Biourine's Effect on Fusarium Wilt in Shallots Adaptive Climate Change

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Abstract:- Climate change can exacerbate the spread and severity of plant diseases, including Fusarium Wilt, due to altered weather patterns and increased stress on plants. This study aims to determine the effect of dosage application of *Trichoderma harzianum* fermented biourine on Fusarium Wilt disease in several shallot varieties. The method used in this research is an experimental method with a factorial completely randomized design consisting of two factors, namely doses of biourine and varieties of shallots. Biourine dosage factors include q0= Without biourine application+ Fusarium sp. inoculation; q1= 5 ml/plant+Fusarium sp. inoculation; q2 = 15 ml/plant+Fusarium sp. inoculation; q3= 25 ml/plant+Fusarium sp. inoculation; and q4= 35 ml/plant+Fusarium sp. inoculation. Varieties factors include v1= Enter Monca and v2 = Bali Karet. There were 10 treatments from a combination of varieties and biourine doses. The results of the study showed that the most effective dose of biourin in suppressing Fusarium Wilt disease in shallot plants was 35 ml (q4). Dose 35 ml of *T. harzianum* fermented biourin showed a significant impact on increasing plant height, number of leaves, and number of shallot plants. The Keta Monca variety showed a better number of shallot plants, while the Bali Karet variety showed better plant height and number of leaves. These findings underscore the importance of using biourine with *T. harzianum* to sustainably manage plant diseases and improve crop yields while selecting resilient varieties like Keta Monca to enhance agricultural productivity and resilience in climate change conditions.

***Keywords**:- Climate Change Adaptatio, Fusarium Wilt, Trichoderma Harzianum, Shallot Varieties.*

I. INTRODUCTION

The primary disease affecting shallots is Fusarium Wilt, caused by the pathogenic fungus Fusarium sp. This disease leads to rapid wilting, root rot, drooping plants, white fungal colonies at the bulb base, yellowing and twisting leaves, and ultimately, plant uprooting due to impaired root growth [1]. Local farmers often rely on chemical fungicides, which can harm the environment and leave residues in agricultural products, posing risks to human health. To mitigate these negative impacts, the use of antagonistic microbes, which can inhibit the development of pathogenic fungi, is being explored [2].

Biological control of Fusarium Wilt can be achieved using biourine fermented with the antagonistic fungus *Trichoderma harzianum*. Biourine is an organic plant fertilizer produced through the anaerobic fermentation of fresh livestock urine with added nutrients and microorganisms [3]. Research by Widyaswari et al. (2017) indicates that the combination of biourine and inorganic fertilizers can enhance the growth and yield of shallots [4]. The antagonistic properties of *T. harzianum* offer an environmentally friendly alternative for pathogen control [5]. Further studies by Sudantha et al. (2020) demonstrated that *T. harzianum* could increase shallot resistance to Fusarium Wilt and improve growth and yield [6]. Similarly, Yusrinawati et al. (2017) found that applying *T. harzianum* bioactivators at a dose of 20 g/plant could boost the yield of wet and dry bulbs by 34.43% and 40.21%, respectively [7].

In the context of climate change, sustainable agricultural practices become even more critical. Climate change can exacerbate the spread and severity of plant diseases, including Fusarium Wilt, due to altered weather patterns and increased stress on plants [8]. This study aims to determine the effect of different doses of *T. harzianum* fermented biourine on Fusarium Wilt disease in two shallot varieties, Keta Monca and Bali Karet, and to evaluate their interaction in reducing disease incidence. The findings could provide insights into sustainable agricultural practices and climate change adaptation strategies for shallot cultivation, helping to ensure stable and resilient crop production in the face of a changing climate.

II. MATERIALS AND METHODS

➢ *Time and Place of Research*

The experiments were conducted from March to June 2023 at the Microbiology Laboratory and Greenhouse, Faculty of Agriculture, University of Mataram.

- *Research Methods*

The experimental method with a completely randomized factorial of two factors, namely biourine dose and variety. Biourin dosage factors include q0 = No biourin application + fungal inoculation *Fusarium* sp.; q1= 5 ml/plant+fungal inoculation *Fusarium* sp.; q2= 15 ml/plant+fungal inoculation *Fusarium* sp.; q3= 25 ml/plant+fungal inoculation *Fusarium* sp.; and q4= 35 ml/plant+fungal inoculation *Fusarium* sp. Variety factors include v1= Keta Monca variety and v2= Bali Karet variety. Ten treatments, combining different varieties and biourin doses, were tested with four replications each, resulting in a total of 40 experimental units.

- *Research Tools and Materials*

The tools used include stationery, autoclave, hoe, bucket, cork-borer, funnel, beaker, haemocytometer, hand counter, hot plate, syringe 10 and 50 cc, ent needle, brush,

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laminar air flow cabinet, bunsen lamp, milling machine, microscope, oven, ruler, tape measure,petri dish, tweezers, dropper pipette, knife, sieve, spatula, erlenmeyer tube, analytical balance, analog scale, and jerry can container. Meanwhile, the materials used include agar, sterile water, distilled water, 70% alcohol, aluminum foil, chloramphenicol, dextrose, absolute ethanol 96%, fungal isolate *Fusarium* sp., pure isolate of fungus *T. harzianum* SAPRO-07, cotton, potato, label paper, plastic wrap, polybags, manure, NPK 16-16-16 fertilizer, coffee leaf litter (*Coffea* sp.), spirits, garden soil, shallot seed bulbs of Keta Monca, Bali Karet varieties, and aerated cow urine.

- *Research Implementation*

The process of making fermented biourin *T. harzianum* starting by collecting coffee plant leaves as a host *T. harzianum*. The collected leaves then undergo a drying process by drying them in direct sunlight. After drying, the leaves are ground into powder using a grinding machine. The next step involves using cow urine that has gone through an aeration process to remove ammonia as a raw material.

The treatment.*Fusarium* sp. suspension was made (spore density 10^6) then inoculated on shallot plants using a syringe around the base of the plant stem or into the soil around the plant. Caring for shallot plants includes watering and weeding. Harvesting is done when the shallot plants reach the age of 60-70 days after planting (DAP).

Observations of disease incidence were made by counting the number of plants affected by the disease starting at 1 weeks after inoculation (WAI) to 4 WAI until the plants entered the generative stage. The calculation of disease incidence is carried out using the following formula:

$$I = \frac{n}{N} x\ 100\ \%$$

Information:

I = Disease incidence;
n = Number of plants showing symptoms;
N = Number of plants observed.

Plant height is measured from the base of the stem to the highest tip using a ruler and meter. The number of leaves was calculated based on the clumps of each sample plant from 1 weeka after planting (WAP) to 7 WAP. Observation of the number of shallot plants was carried out by counting the number of shallot plants that grew from 5 WAP to 8 WAP.

III. RESULTS AND DISCUSSION

In further test results in Table 1, it can be seen that the biourine treatment dose of 35 ml (q4) produced the highest average plant height in the age period 1-7 WAP. At the age of 1 WAP, the average height of plants that received a biourine dose of 35 ml reached 6.02 cm, which was significantly different from other biourine dose treatments. It is possible that this is caused by the abundant macro and micro content in biourine, which is a source of nutrients that are really needed by plants. In addition, it is suspected that biourine contains growth hormones such as auxin, cytokinin and gibberellin in sufficient concentrations to stimulate plant growth and development [9].

Further, the presence of *T. harzianum* in biourine is also believed to stimulate plant growth. According to Martines et al. (2014), Trichoderma can produce plant growth regulators (PGRs) such as auxin, IAA, salicylic acid (SA), jasmonic acid (JA), and 1-aminocyclopropane-1-carboxylate (ACC), a precursor of ethylene [10]. The results of this research are supported by Kalay et al. (2019), who found that Trichokompos containing *T. harzianum* not only acts as a biocontrol agent for plant diseases but also positively impacts the growth of tomato seedlings [11]. Similarly, Astiko and Muthahanas (2019) observed that the application of Streptomyces sp. and Trichoderma sp. resulted in the best plant height [12]. Amin et al. (2015) also reported positive responses in shoot height, fresh and dry shoot weight, root dry weight, and root length, highlighting the role of *T. harzianum* in enhancing rooting, growth, and plant yield [13]. Additionally, Marianah (2013) stated that *T. harzianum* plays a role in decomposing soil organic matter, which contains essential nutrients such as N, P, S, and Mg that support plant growth [9]. Therefore, *T. harzianum* in biourine may also contribute to the availability of nutrients for plant growth.

Table 1 Further Test Results for Average Height of Shallot Plants

Treatment	Plant Height (cm)						
	1 WAP	2 WAP	3 WAP	4 WAP	5 WAP	6 WAP	7 WAP
Dosis biourin							
q0	$3,68^{b}$	$7,78^{b}$	$9,86^{b}$	$14,92^{b}$	$21,60^{b}$	$29,76^{b}$	$38,88^{b}$
q1	$3,92^{b}$	$8,21^{b}$	$10,34^{b}$	$14,70^{b}$	$24,64^{ab}$	$37,91^{a}$	$46,27^{ab}$
q2	$3,98^{b}$	$10,05^{ab}$	$12,65^{b}$	$22,52^{a}$	$29,99^{a}$	$39,16^{a}$	$48,99^{a}$
q3	$3,96^{b}$	$9,68^{ab}$	$13,01^{ab}$	$21,11^{a}$	$28,01^{ab}$	$34,02^{ab}$	$47,08^{ab}$
q4	$6,02^{a}$	$13,93^{a}$	$17,40^{a}$	$22,86^{a}$	$30,10^{a}$	$38,44^{a}$	$49,39^{a}$
HSD 5%	1,133	3,089	3,193	3,082	4,619	5,509	5,856
Variety							
v1	$2,70^{b}$	$5,26^{b}$	$7,39^{b}$	$11,88^{b}$	$18,60^{a}$	$28,07^{b}$	$37,59^{b}$
v2	$5,92^{a}$	$14,60^{a}$	$17,92^{a}$	$26,57^{a}$	$35,14^{a}$	$43,65^{a}$	$54,66^{a}$
HSD 5%	0,716	1,954	2,019	1,949	2,921	3,484	3,703

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* Note: Numbers in Each Column Followed by the Same Letter Are not Significant in the HSD Test At The 5% Level. WAP = Week After Planting.

In the context of climate change, these findings are particularly significant. Climate change can lead to increased stress on plants and alter disease dynamics, making it essential to find sustainable agricultural practices that enhance plant resilience. The ability of *T. harzianum* to produce plant growth regulators and decompose organic matter can help plants better with environmental stresses associated with climate change. At 2-7 WAP, treatment with a dose of 35 ml of biourine showed no significant differences in plant height compared to other treatments, indicating that all treatments had equal potential in increasing plant height. This suggests that *T. harzianum* in biourine can be an effective strategy for maintaining plant growth and health in the face of climate change [14].

Turning to varieties, the results in Table 1 show that the Bali Karet variety (v2) consistently exhibits the best plant height at 1-7 WAP. The Bali Karet variety demonstrated significant differences in plant height compared to the Keta Monca variety (v1). These findings align with research by Sudantha et al. (2023), which indicates that the Bali Karet variety has greater plant height than the Ampenan and Keta Monca varieties [15]. Sudantha et al. (2018) also reported that the average plant height of the Bali Karet variety was 50-60 cm, significantly higher than the Ampenan and Keta Monca varieties, which reached 26-45 cm and 26-46 cm, respectively [16]. This difference is attributed to genetic factors and the variety's ability to adapt to environmental conditions such as sunlight intensity, irrigation, rainfall, and soil conditions [17] (Sudantha et al., 2018). According to Elvira et al. (2015), differences in plant growth and production are influenced by internal factors such as genes and hormones, which regulate growth through inherited traits [18].

Climate change can alter environmental conditions, affecting sunlight intensity, rainfall patterns, and soil moisture levels, all of which influence plant growth. The Bali Karet variety's superior ability to adapt to varying environmental conditions makes it a valuable asset in the face of climate change. Its genetic resilience can help ensure stable yields even as climate patterns become more unpredictable. By selecting and cultivating varieties like Bali Karet that demonstrate robust growth and adaptability, farmers can better mitigate the impacts of climate change on agricultural productivity.

Table 2 Further Test Results for the Average Number of Leaves of Shallot Plants

Treatment	**Number of Leaves**						
	1 WAP	**2 WAP**	**3 WAP**	**4 WAP**	**5 WAP**	**6 WAP**	**7 WAP**
Dosis biourin							
q0	1,95^{b}	3,65^{c}	4,9^{b}	5,77^{c}	7,59^{c}	11,11^{b}	13,64^{c}
q1	2,49^{ab}	3,98^{bc}	5,94^{ab}	6,82^{bc}	8,42^{bc}	11,73^{b}	14,12^{bc}
q2	2,72^{a}	4,73^{ab}	6,43^{a}	7,66^{b}	9,69^{a}	12,06^{ab}	15,33^{abc}
q3	3,00^{a}	4,58^{ab}	6,46^{a}	7,82^{ab}	9,50^{ab}	12,73^{ab}	15,52^{ab}
q4	3,03^{a}	5,12^{a}	7,01^{a}	9,04^{a}	10,54^{a}	13,59^{a}	15,93^{a}
HSD 5%	0,494	0,649	0,831	0,884	0,877	1,179	1,232
Variety							
v1	1,51^{b}	3,18^{b}	4,62^{b}	5,85^{b}	7,82^{b}	11,17^{b}	13,27^{b}
v2	3,77^{a}	5,65^{a}	7,68^{a}	9,00^{a}	10,47^{a}	13,32^{a}	16,54^{a}
HSD 5%	0,313	0,411	0,525	0,559	0,554	0,746	0,779

* Note: Numbers in each column followed by the same letter are not significant in the HSD test at the 5% level. WAP = Week After Planting.

Further test results in Table 2 revealed that a biourine dose of 35 ml (q4) resulted in the highest average number of leaves in the age range of 1-7 weeks after planting (WAP). Research by Sumini (2022) supports this finding, indicating that biourine application significantly impacts leaf number due to its nutrient-rich composition, which is easily absorbed by plants and aids in water absorption [19]. A plant's ability to absorb water effectively enhances nutrient uptake, leading to an increase in the number of leaves. Additionally, Sudana et al. (2013) noted that biourine contains high concentrations of growth hormones such as auxin, cytokinin, and gibberellin, which stimulate plant growth and development [8]. Nurita and Yuliani (2023) demonstrated that the synergy between auxin and gibberellin hormones could increase the number of leaves in eggplant plants [20]. The consistent results across different biourine doses suggest that all treatments have the potential to increase the number of leaves.

Regarding the variety factor, the Bali Karet variety (V2) had the highest average number of leaves at 1-7 WAP. Treatment with the Bali Karet variety showed a significant difference compared to the Keta Monca variety (V1) in terms of leaf number. This finding aligns with research by Sudantha et al. (2023), which reported that the Bali Karet variety produced 50-55 leaves, more than the Ampenan variety (45-50 leaves) and the Keta Monca variety (17-47 leaves) [15]. These differences are attributed to genetic factors and the variety's adaptability to environmental conditions, such as sunlight intensity, soil moisture, rainfall, and soil conditions [16].

These findings highlight the importance of selecting and cultivating plant varieties and treatments that enhance resilience and adaptability [21]. Climate change can

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exacerbate environmental stresses, affecting water availability, nutrient uptake, and overall plant health. Biourine, with its rich nutrient content and growth-stimulating hormones, can help plants better withstand these stresses by improving water and nutrient absorption. Additionally, varieties like Bali Karet, which demonstrate superior adaptability and growth under varying conditions, can essnsure stable yields and contribute to sustainable agricultural practices. By integrating biourine treatments and resilient plant varieties, farmers can better cope with the challenges posed by climate change.

Table 3 Results of Further Tests for the Average Number of Shallot Plants

Treatment	Number of Cubs			
	5 WAP	6 WAP	7 WAP	8 WAP
Dosis biourin				
q0	1.17^{b}	2,35^{b}	4,05^{c}	7,52^{b}
q1	1,71^{ab}	2,47^{b}	4,43^{bc}	8,19^{ab}
q2	1,80^{a}	2,79^{b}	5,16^{b}	8,31^{ab}
q3	1,86^{a}	3,17^{ab}	5,36^{ab}	8,65^{ab}
q4	2,12^{a}	4,03^{a}	6,23^{a}	9,83^{a}
HSD 5%	0,423	0,671	0,742	1,398
Variety				
v1	2,03^{a}	3,76^{a}	7,37^{a}	11,69^{a}
v2	1,43^{b}	2.16^{b}	2,72^{b}	5,30^{b}
HSD 5%	0,268	0,424	0,469	0,884

- Note: Numbers in each column followed by the same letter are not significant in theHSD test at the 5% level. WAP = Week After Planting.

Based on the results presented in Table 3, the control group with a biourine dose of 0 ml showed the lowest number of off spring, indicating that the administration of fermented biourine with *T. harzianum* has the potential to increase the number of tillers in shallot plants. At the age of 5-8 WAP, a biourine dose of 35 ml (q4) resulted in the highest average number of off spring. This can be attributed to the presence of *T. harzianum* in biourine, which is thought to stimulate an increase in the number of tillers in shallots. This finding is consistent with research by Sudantha et al. (2023), which stated that the application of a stimulator containing *T. harzianum* can increase the number of tillers and the weight of dry onion bulbs at harvest [15]. Biourine is believed to contain high levels of nitrogen (N), which makes plants greener, supports the photosynthesis process optimally, and positively impacts the quality and quantity of the final harvest. Sufficient nitrogen content stimulates the growth of seedlings, subsequently affecting the number of tubers produced. This aligns with Wahyu (2013) explanation that higher nitrogen content stimulates seedling growth, contributing to an increase in the number of tubers [22].

At the age of 5-8 WAP, treatment with a biourine dose of 35 ml showed results that were not significantly different in terms of the number of off spring, indicating that all treatments had the same potential to increase the number of off spring. Concurrently, further test results in Table 3 show that the Keta Monca (V1) variety gave the highest average number of shallot plants at the age of 5-8 WAP. This finding is confirmed by research by Sudantha et al. (2023a), which indicates that the Keta Monca variety shows the best performance in terms of the number of tillers [23].

Climate change can lead to more frequent and severe environmental stresses, such as droughts and nutrient depletion, which can negatively impact crop yields [24]. The use of biourine enriched with *T. harzianum* can help mitigate these stresses by enhancing the nutrient content and growth hormone levels in the soil, promoting healthier and more resilient plant growth. Additionally, selecting shallot varieties like Keta Monca, which demonstrate superior tillering performance, can further improve crop yields under changing climatic conditions. By integrating biourine treatments and resilient crop varieties, can develop more sustainable agricultural practices that enhance crop resilience and productivity in the face of climate change

Table 4 Results of Further Tests for Average Incidence of Shallot Plant Diseases

Treatment	Disease Incidence (%)			
	1 WAI	2 WAI	3 WAI	4 WAI
Dosis biourin				
q0	5,22^{a}	6,89^{a}	7,72^{a}	7,89^{a}
q1	2,62^{b}	3,33^{b}	3,78^{b}	4,49^{b}
q2	1,66^{b}	2,14^{bc}	2,59^{bc}	3,78^{bc}
q3	0,71^{b}	1,19^{bc}	1,66^{bc}	2,62^{bc}
q4	0,71^{b}	0,71^{c}	0,71^{c}	1,19^{c}
HSD 5%	1,407	1,615	2,091	2,176

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* Note: Numbers in each column followed by the same letter are not significant in theHSD test at the 5% level. WAI = Week After Inoculation.

Based on the results in Table 4, the control group with a biourine dose of 0 ml showed the highest level of disease incidence, indicating that the application of fermented biourine with *T. harzianum* has the potential to suppress Fusarium Wilt disease in shallot plants. A biourine dose of 35 ml (q4) demonstrated the lowest percentage of Fusarium Wilt disease incidence. This can be attributed to the presence of *T. harzianum* in biourine as a biological agent that can effectively suppress Fusarium Wilt. Research by Sudantha et al. (2018) shows that the application of liquid legundi leaf extract bioactivators and tablets containing Trichoderma spp. can suppress the occurrence of Fusarium Wilt and increase shallot yields [16]. Rahmawati (2023) also supports this finding, stating that *T. harzianum* dosing effectively reduces the number of leaves affected and the intensity of disease attacks in shallot plants infected with Fusarium disease [25]. Sulistiono (2014) noted that all *T. harzianum* isolates produce the chitinase enzyme, which can reduce phytopathogenic activity and result in a reduction in disease intensity [26], as explained by Kalay et al. (2023) [27]. Additionally, research by Sudantha et al. (2021) highlights *T. harzianum*'s role in controlling pathogenic fungi through three mechanisms: acting as mycoparasites causing lysis and crystallization of pathogenic fungal hyphae, producing antibiotics toxic to pathogens, and quickly competing for nutrients and space [28].

Climate change can exacerbate plant disease incidence and severity due to altered weather patterns and increased environmental stress [29]. Using biourine enriched with *T. harzianum* offers a sustainable solution to manage plant diseases like Fusarium Wilt. By enhancing the biological control mechanisms in the soil, *T. harzianum* helps reduce the dependency on chemical fungicides, which can have harmful environmental impacts [30]. Moreover, promoting plant health and resilience through biological agents like *T. harzianum* can lead to more stable and productive agricultural systems amidst the challenges posed by climate change.

The morphology of *T. harzianum* (Figure 1) consists of conidia located on conidiophore structures. The conidiophores can branch in a pyramid shape with repeated lateral branches, while the branches become shorter towards the tip. The phialides/hyphal branches are slender and long, especially at the apex of the branches, measuring 18 x 2.5 µm. The conidia are semirounded to short oval, measuring (2.8-3.2) x (2.5-2.8) µm and have smooth walls. At the beginning of mycelial growth, it appears as cotton-like white growth, which later turns green and then dark green. On the seventh day, the mycelial growth has filled the plate. The mycelium continues to grow and forms a ring-like structure after fully colonizing the plate [31]. The pattern and shape of the conidial wall are rough and subglobose, with a green to dark green color. Green conidia production is observed during the growth of the isolate, and it is denser in the center with dark green conidia spread across the culture, along with the formation of 1-2 concentric rings around the culture [32]. According to Gusnawaty et al. (2014), the conidiophore of *T. harzianum* is erect, branched, with short phialides, thicker, and oval-shaped conidia [33]. The colony on PDA medium is dark green and circular in shape. The colony diameter reaches over 9 cm within 5 days. The characteristics of this isolate reflect those of *T. harzianum*.

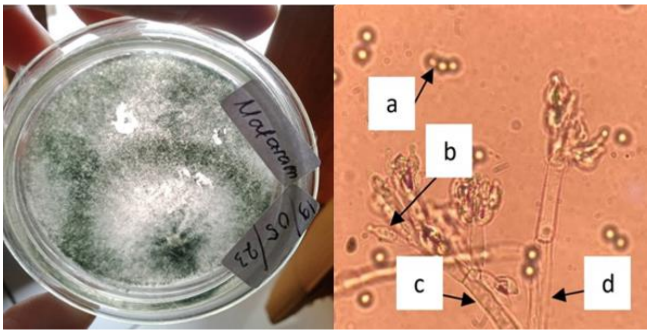

Fig 1 Trichoderma sp.; (a) Conidium, (b) Conidiophore, (c) Hypha, (d) Stigma.

IV. CONCLUSION

The most effective dose of biourin in suppressing Fusarium Wilt disease in shallot plants was 35 ml (q4). Dose 35 ml of T. harzianum fermented biourin showed a significant impact on increasing plant height, number of leaves, and number of shallot plants. The Keta Monca variety showed a better number of shallot plants, while the Bali Karet variety showed better plant height and number of leaves. These findings underscore the importance of using biourine with T. harzianum to sustainably manage plant diseases and improve crop yields while selecting resilient varieties like Keta Monca to enhance agricultural productivity and resilience in climate change conditions

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