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Effect of Fobio on Intensity of Moler Disease (*Fusarium oxysporum*) on Various Shallot Cultivars

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ABSTRACT

Fusarium oxysporum is the pathogen that causes moler disease which can reduce the productivity of shallot plants. The use of cultivars greatly affects the production. Control method that is more environmentally friendly is currently being pursued towards organic farming. Fobio is a biopesticide containing plant root microorganisms as biological control agents. This study aims to determine the effect of Fobio biopesticide on Fusarium wilt (moler) disease incidence and production of three shallot cultivars. The experiment was conducted in the shallot planting area of Sukorejo Village, Rejoso District, Nganjuk Regency, East Java. This experiment was arranged in the split plot design with two factors. The main plot of the concentration of the biopesticide formula (Fobio) consisted of 3 levels namely control (chemical fungicide), 5 ml/liter, and 10 ml/liter, and the subplot of shallot cultivar consisted of 3 types namely Tajuk, Biru Lanchor, and Super Philip. The observation in this study were the incubation period, disease symptoms, disease intensity, and shallot bulb yield. The results showed that Fobio biopesticide at the concentration of 10 ml/liter caused the same disease suppression as chemical fungicide (control) in the three shallot cultivars with the disease incidence of 18.78%. Fobio biopesticide concentration of 10 ml/liter had also the same effect as chemical fungicide on the production of those shallot cultivars, with the average wet weight of 2.25 kg/treatment and dry weight of 1.49 kg/treatment.

Keywords: Biopesticide Formulation, Disease incidence, Fusarium basal rot, Fusarium wilt, leaf twisting

Pengaruh Formulasi Biopestisida terhadap Intensitas Penyakit Moler (Fusarium oxysporum) pada Berbagai Kultivar Bawang Merah

ABSTRAK

Fusarium oxysporum adalah patogen penyebab penyakit moler yang dapat mengakibatkan menurunnya produktivitas tanaman bawang merah. Penggunaan kultivar sangat berpengaruh terhadap produksi. Pengendalian yang lebih ramah lingkungan saat ini sangat diupayakan untuk menuju pertanian organik. Fobio adalah biopestisida yang mengandung mikroorganisme akar tanaman sebagai agen pengendali hayati. Penelitian ini bertujuan untuk menguji pengaruh biopestisida Fobio terhadap insidensi penyakit layu Fusarium (moler) pada tiga kultivar bawang merah, serta produksinya bawang merah. Percobaan dilaksanakan di lahan pertanaman bawang merah Desa Sukorejo, Kecamatan Rejoso, Kabupaten Nganjuk, Jawa Timur. Percobaan ini menggunakan rancangan petak terbagi dengan dua faktor. Petak utama adalah konsentrasi formula biopestisida (Fobio), terdiri dari 3 taraf yaitu kontrol (aplikasi fungisida sintetik tanpa Fobio), 5 ml/liter, dan 10 ml/liter. Anak petak adalah kultivar bawang merah yang terdiri dari 3 kultivar yaitu Tajuk, Biru Lanchor, dan Super Philip. Pada percobaan ini diamati periode inkubasi, gejala penyakit, intensitas penyakit, dan hasil umbi bawang merah. Hasil penelitian menunjukkan biopestisida Fobio dengan konsentrasi 10 ml/liter memiliki kemampuan yang sama dengan fungisida sintetik dalam menekan insidensi penyakit layu pada ketiga kultivar bawang merah yang diuji, dengan nilai insidensi penyakit sebesar 18,78%. Biopestisida Fobio konsentrasi 10 ml/liter juga mampu menghasilkan produksi yang setara dengan aplikasi fungisida sintetik pada ketiga kultivar bawang merah tersebut dengan rata-rata berat basah 2,25 kg/perlakuan dan berat kering 1,49 kg/perlakuan.

Kata Kunci: Formulasi biopestisida, busuk basal fusarium, Insidensi penyakit, layu fusarium, daun melilit

INTRODUCTION

Shallot (*Allium ascalonicum* L.) is a widely cultivated vegetable plant in Indonesia because it has high productivity and economic value. According to the Central Bureau of Statistics (Badan Pusat Statistik, 2020) shallot production in Indonesia in 2019 was 1.580.247 tons, and in 2020 it increased by14,9% so that the production was 1.815.445 tons. The shallot cultivation process is inseparable from various obstacles, one of which is related to moler disease caused by the fungus *F. oxysporum*. This fungus may decrease the production of up to half of the potential yield and even cause crop failure (Juwanda *et al*., 2016). *F. oxysporum* is a fungus that lives as saprophyte in the soil and quite difficult to control. This pathogen has a fairly wide host range and can infect plants from seedling to mature plants (Suryaminarsih and Mujoko, 2020). Moler is a local native designation of the disease symptoms caused by *F. oxysporum* on shallot plants in the form of wilted accompanied by twisted leaves. Another symptom is that the leaf tip of the shallot is brownish yellow. These wilted leaves will then fall to the ground (Hartati *et al.*, 2022). Moler disease is also known as fusarium wilt, fusarium basal rot, leaf twisting, and other terms, according to the disease symptoms and locality. Factor affecting the growth and development of *F. oxysporum* is humid condition such as in overly dense gardens or planting areas, especially during the rainy season many new infections occur. *F. oxysporum* lives in the soil and can last a long time in its environment (Nugraheni, 2010).

The use of cultivars greatly affects the production because they have different adaptation levels in each area (Kartinaty *et al*., 2018). There are some cultivars that are able to adapt to lowland areas, namely Super Philip and Tajuk. Likewise with the Biru Lanchor which is usually cultivated in lowland areas (Jasmi *et al*., 2013; Kasim *et al*., 2021). Super Philip and Biru Lanchor cultivars are often planted by farmers because they have a high yield quantity, but these cultivars are not resistant to moler disease (Wiyatiningsih *et al*., 2009).

The farmers generally control moler disease by using synthetic chemical fungicides. Plants treated with chemical fungicides will contain chemical residues which are of course very harmful to the health of people who consume them (Aini, 2018; Pratiwi, 2018). Control method that is more environmentally friendly is currently being pursued towards organic farming, which has begun to be implemented in Indonesia. A biopesticide formula with the trade name Fobio is an environmentally friendly biopesticide product, containing plant root microorganisms as biological control agents, including phosphate solubilizing bacteria, photosynthetic bacteria, amylolytic bacteria, proteolytic bacteria, *Rhizobium* sp., *Lactobacillus* sp., ammonifying bacteria, and nitrifying bacteria (Wiyatiningsih and Sukaryorini, 2009).

The bacteria contained in this Fobio biopesticide formula are able to reduce disease inoculums, suppress disease development, and increase plant resistance. In addition, Fobio can optimize growth and increase plant productivity. Research on this biopesticide formula has already been carried out in a greenhouse scale. The results of the study by Wiyatiningsih and Sukaryorini (2009) showed that the application of Fobio in the greenhouse scale by spraying method at the concentration of 2.5 ml/liter was able to increase the resistance of shallots to *F. oxysporum*. This study is a follow-up research carried out in the field, by using higher concentrations of the biopesticide, because it is carried out in open land with more risks, which is related to the environment. The concentrations of the Fobio biopesticide used were 5 ml/liter and 10 ml/liter, twice and four times of the greenhouse experiment result. This study aimed to determine the effect of the concentration of Fobio biopesticide on *Fusarium* wilt disease incidence (moler disease) in Tajuk, Super Philip and Biru Lanchor shallot cultivars, as well as on the shallot production.

MATERIALS AND METHODS

The experiment was conducted in Sukorejo Village, Nganjuk, East Java, from September to November 2022. The materials used were shallot cultivar Tajuk, Super Philip, and Biru Lanchor, a fungicide (mancozeb) as a control or comparison treatment, as well as the biopesticide formulation with the trademark Fobio. Fobio contains microorganisms originating from the rhizosphere of coconut (*Cocos nucifera* L.), mangrove (*Rhizophora racemos*), sugar cane roots (*Saccharum officinarum* L.), and siwalan (*Borassus flabellifer*), with the carrier medium in the form of potato extract, sugar, black sticky rice extract, and beef extract (Wiyatiningsih, 2009).

This study was arranged in the split plot design with two factors. The main plot was the concentration of the biopesticide formulation (Fobio) consisted of 3 levels, namely control (mancozeb), 5 ml/liter, and 10 ml/liter. The subplot was shallot cultivars consisted of 3 types, namely Tajuk, Super Philip, and Biru Lanchor. This design resulted in 9 treatment combinations and was repeated 3 times to obtain 27 experimental units. The treatments used were: K0BT (mancozeb + Tajuk), K0BB (mancozeb + Biru Lanchor), K0BP (mancozeb + Super Philip), K5BT (5 ml/liter Fobio + Tajuk), K5BB (5 ml/liter Fobio + Biru Lanchor), K5BP (5 ml/liter Fobio + Super Philip), K10BT (10 ml/liter Fobio + Tajuk), K10BB (10 ml/liter Fobio + Biru Lanchor), K10BP (10 ml/liter Fobio + Super Philip). Obtained data were then analyzed using analysis of variance (ANOVA). If the F test shows a significant effect, then it is continued with the Duncan Multiple Range Test at a significant level of 5%.

Land Preparation

Land preparation was carried out by dividing the land into 27 plots and adding 2 kg of chicken manure per plot. Furthermore, soil sterilization was carried out by spraying Fobio with a concentration of 10 ml/liter of water at a dose of 2 liters of suspension/3m^2. Spraying was only carried out in the treatment plots with the concentration of 5 ml/liter and 10 ml/liter 3 times with an interval of 3 days. Control treatment without Fobio application.

Fobio Biopesticide Formula Application

There were 3 cultivars of shallot seeds used, namely Tajuk, Super Philip, and Biru Lanchor. Seed preparation at treatment of Fobio concentration was carried out by soaking the seeds in the biopesticide formula with a concentration of 10 ml/liter of water for 60 minutes before planting. Meanwhile, the control treatment was carried out without soaking. Planting was done using a spacing of 20 X 15 cm. Shallot seeds planted for one treatment (each plot) were 100 seeds. Fobio application was also carried out during maintenance after the planting process by spraying the shallot plants 6 times or until harvest with an interval of 7 days. The K5 and K10 treatments were carried out by applying the biopesticide formula. The K5 treatment was spraying with concentration of 5 ml/liter of water, while the K10 treatment was spraying with concentration of 10 ml/liter of water. Meanwhile, the K0 (control concentration treatment) was spraying a fungicide with the active ingredient mancozeb (Detazeb) at a concentration of 2g/liter. The dose used in all treatments was the same, namely 2 liters of suspension/ $3m^2$. Spraying the fungicide and Fobio 6 times or until harvest with an interval of 7 days.

Plant Maintenance

Shallot plant maintenance included supplementary fertilization, watering, and weeding. The first follow-up fertilization at 10 days after planting (DAP) as much as 300 g/plot of NPK fertilizer. The second follow-up fertilization was carried out at 20 DAP by applying 300 g/plot of NPK fertilizer. The third follow-up fertilization at 35 DAP by applying 250 g/plot of Grower fertilizer. Watering was done 2 times a day, in the morning and evening or according to the needs of the soil/plant. Weeding was done when weeds started to grow around the shallot plants or adjusted to conditions.

Observation Parameters

Incubation Period

Observation of moler symptoms during the incubation period were carried out every day starting from the shallot planting until the plants showed first symptoms of moler disease.

Symptoms of Moler Disease

This observation was carried out when the plants showed the initial symptoms such as pale green leaf color, elongated leaves and twisting. The plant part observed was the leaves, especially from the physical shape and color of the leaves.

Disease Incidence

Calculation of the disease incidence caused by the pathogen *F. oxysporum* on shallots was carried out from planting to harvest using a systemic formula. Symptoms observations were made once a week for about two months on the shallot plantations from the first time the symptoms appeared until harvest. Adapted from Nurhayati (2011) the formula for the incidence of systemic disease is as follows:

$$I = \frac{a}{b} \text{ x } 100\ \% \qquad \ldots (1)$$

Description :

I = Disease incidence
a = Total of infected plants
b = Total planted plants

The disease incidence can be categorized as shown on Table 1 (Prasetyo *et al.*, 2017):

Table 1. Categories of Disease Incidence

No.	Disease Incidence	Damage level
1	< 11 %	Less (L)
2	$11 < X \leq 25\%$	Mild (M)
3	$25 < X \leq 75\ \%$	Heavy (H)
4	$75 < X \leq 100\ \%$	Very Heavy (VH)

Shallot Production

Harvesting was done at 51 DAP or when the plants already showed physical characteristics that they were ready to be harvested. The physical characteristics of shallots ready for harvest include showing 80% of the leaves falling, the neck of the stem is empty, the bulbs are sticking out to the ground and already purplish-red in color. Observation of bulb production was carried out during post-harvest by weighing the fresh bulb weight and the marketable dry bulb weight of shallot. The bulbs were dried for 7 to 14 days in the sun.

RESULTS AND DISCUSSION

Incubation Period

The results of the analysis of variance showed that the combination treatment and each treatment factor did not have significant effects on the incubation period variable. This was because the time of appearance of symptoms in all treatments were almost the same or close together.

Figure 1 shows that the highest average incubation period was found in the control combination treatment with Tajuk cultivar (K0BT), which was 29 DAP (Day After Planting). Although all treatments had statistically the same average incubation periods. The level of incubation period in the treatment concentration of 10 ml/liter (23 DAP) statistically the same with the control treatment (28 DAP). This indicated that the application of biopesticide formula with the concentration of 10 ml/liter and 5 ml/l has the same ability as the chemical fungicide. According to Rojikin *et al* (2021) Fobio biopesticide contains microorganisms that can inhibit the appearance of disease symptoms. These microorganisms are capable of producing secondary metabolites that act as antimicrobials such as lactic acid, acetic acid, hydrogen peroxide, amylase and protease enzymes which also act as protectors against pathogenic infections. According to Hasyidan *et al.* (2021) the length of the incubation period depends on the resistance level of a plant. The longer or slower the incubation period indicates that the plant has a higher level of resistance. Otherwise, the shorter or faster the incubation period indicates that the plant has a fairly low level of resistance.

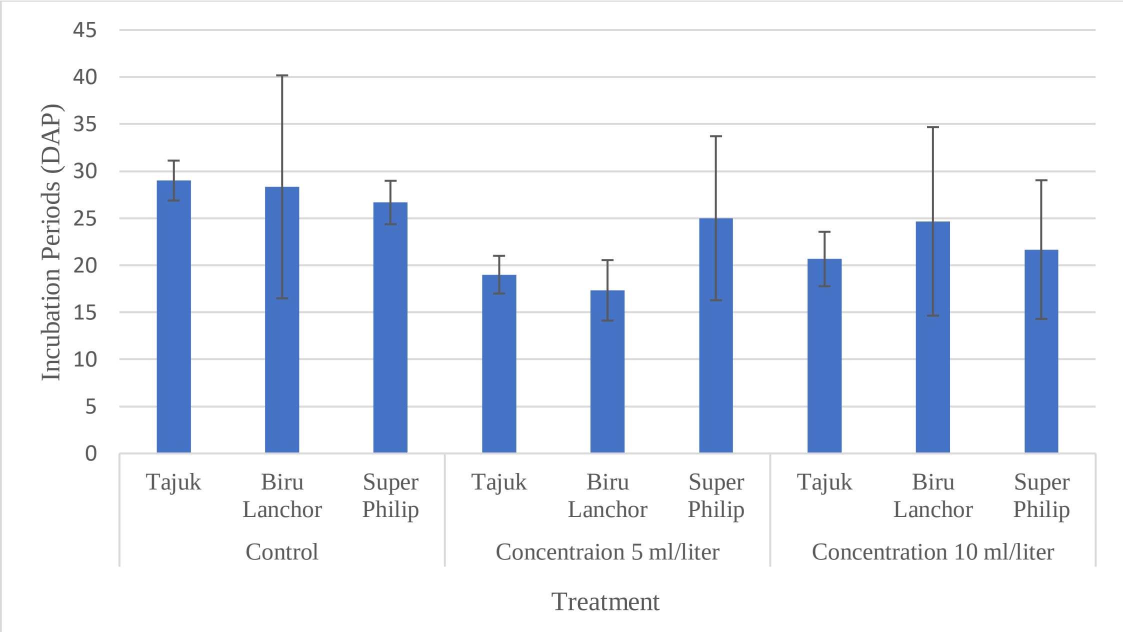

Fig. 1. Bar chart of the average incubation period (DAP)

Symptoms of Moler Disease

The initial symptom of moler disease in shallot plants is that the plant grows with elongated and twisted leaves, starting from the base. In addition, the color of the leaves becomes pale green, slightly yellowish. Advanced symptoms of this disease are wilted and fallen leaves, and gradually the plants will die (Fig. 2.).

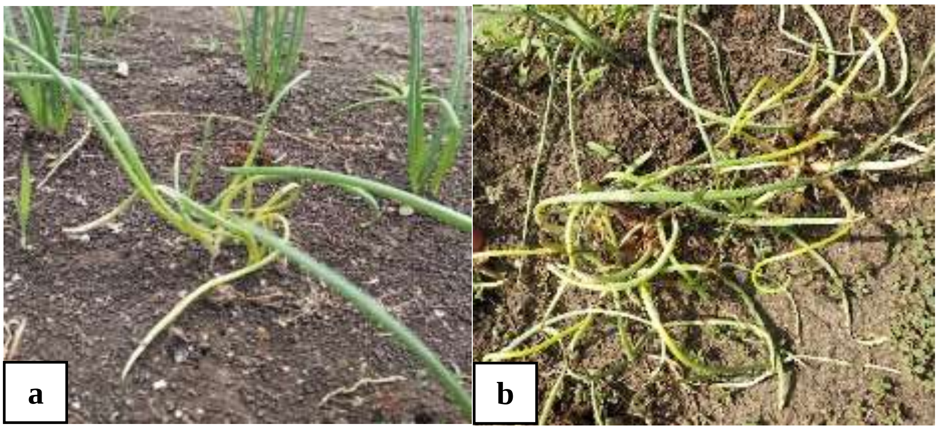

Fig. 2. Moler Symptoms, (a) Early symptoms of leaf curl (b) Further symptoms of fallen and wilted plants.

Symptoms that appear on plants affected by moler pathogen include pale green leaf color, elongated and twisted leaves, and slightly wilting (Prakoso *et al*., 2016). Affected plants will begin to wilt from the lower leaves or the base of leaves to the tip of the leaves. The leaves are originally pale green to yellowish to brownish. The wilt symptom may spread to the whole plant so that the plant falls to the ground (Kaary et al., 2022). According to Isniah and Widodo (2015) advanced symptoms of moler disease on shallot plants are that the plants will dry out and die. Diseased plants will produce smaller and decayed bulbs.

Disease Incidence

Disease incidence is one of the variables used to determine the severity of disease in a plant population. The results of the analysis of variance in disease incidence showed that the combination treatment had no significant effect on each observation, while the concentration factor treatment showed a significant effect on the 35 DAP and 42 DAP observations, and the cultivar factor treatment showed a significant effect on the 35 DAP observations.

Table 2. Moler Disease Incidence in Single Factor Treatment of Biopesticide Concentration

Concentration	Average moler disease incidence (%)			
	21 DAP	28 DAP	35 DAP	42 DAP
Kontrol	0.11 ± 0.33 a	0.67 ± 0.71 a	0.67 ± 0.71 a	1.22 ± 1.30 a
5 ml/liter	2.44 ± 1.74 a	3.78 ± 2.44 a	17.33 ± 16.68 b	27 ± 24.45 b
10 ml/liter	2.78 ± 3.70 a	4.56 ± 4.10 a	13.78 ± 13.67 b	18.78 ± 16.71 ab
DMRT 5%	ns (S1 = 4.17 S2 = 4.32)	ns (S1 = 4.08 S2 = 4.22)	S1=10.60 S2=10.98	S1=19.15 S2=19.85

Explanation : numbers followed by the same letter in one column were not significantly different at the 5% level

Table 2. shows that Fobio at the concentration of 10 ml/liter had the same ability as the use of chemical fungicides in suppressing the development of moler disease, but this Fobio is more environmentally friendly than chemical fungicides. Rahayu *et al* (2021) mentioned that biopesticides containing rhizosphere microorganisms function as PGPR (Plant Growth Promoting Rhizomicroorganism) which can induce systemic acquired resistance (SAR) of a plant. Plants that have received systemic resistance induction are capable of forming chemical compounds that can be used for defense against pathogen attack. Rhizospheric microorganisms that live around plant roots stimulate root cells to produce toxic compounds that can inhibit the growth of pathogens, and induce plant resistance to pathogens.

Table 3. Moler Disease Incidence in Single Factor Treatment of Cultivar

Cultivar	Average moler disease incidence (%)			
	21 DAP	28 DAP	35 DAP	42 DAP
Tajuk	1.78 ± 1.86 a	3.22 ± 2.86 a	4.56 ± 3.61 a	7 ± 5.59 a
Biru Lanchor	2.33 ± 3.67 a	3.56 ± 4.13 a	20 ± 20.78 b	26 ± 27.22 a
Super Philip	1.22 ± 1.99 a	2.22 ± 2.54 a	7.22 ± 6.40 a	14 ± 17.01 a
DMRT 5%	ns (S1 = 1.92 S2 = 2.01)	ns (S1 = 2.71 S2 = 2.84)	S1=10.23 S2=10.71	ns (S1 = 15.51 S2 = 16.23)

Explanation : numbers followed by the same letter in one column were not significantly different at the 5% level DMRT.

Based on Table 3, the type of cultivar also affects the high or low incidence of the disease at 35 DAP. Edi (2019) reported that Tajuk cultivars had advantage for being able to adapt to both the lowlands and highlands, during the dry and rainy season. In addition, this cultivar had a fairly high level of resistance to moler disease. According to Kartinaty *et al.* (2018) various cultivars have different adaptation levels in each development area. Baswarsiati *et al.* (2015) reported that Tajuk cultivar is a local cultivar from Nganjuk which was released by the Minister of Agriculture and submitted by the East Java Agricultural Technology Study Center service from 2000 to 2011. Therefore, the Tajuk cultivar is very suitable if it is cultivated in Nganjuk, East Java because of its favorable environment support for growth.

According to Wiyatiningsih (2011) the factors that influence the level of incidence of moler disease include the condition of the land, the season, and the cultivars planted. Cultivars have different susceptibilities that can cause different levels of damage. Biru Lanchor cultivar had the highest level of disease incidence when it was planted in Nganjuk paddy fields because it has a vertisol soil type. Vertisols are a type of soil with a heavy clay texture, so they have more micropores which cause slow water movement. This condition causes water to be retained around the roots, so that the growth of the roots becomes disturbed and they are more susceptible to pathogens.

Shallots Production

ANOVA result of wet and dry weight of shallot bulbs showed that the combination treatment and each treatment factor did not show any significant effect. The weight of shallot obtained was influenced by several factors during planting. These factors include the type of cultivar planted, environmental conditions, and maintenance.

Based on table 4. the highest wet weight of shallot bulbs was produced in the control combination treatment with Tajuk cultivar (K0BT) at 3.00 kg per treatment, while the lowest in the combination treatment of 5 ml/liter Fobio with Biru Lanchor cultivar (K5BB) at 1.70 kg per treatment. Likewise, the highest bulb dry weight was produced in the control combination treatment with Tajuk cultivar (K0BT) at 2.10 kg per treatment, and the lowest in the combination treatment of 5 ml/liter Fobio with Biru Lanchor cultivar (K5BB) at 1.17 kg per treatment. The shallot production was not maximal, due to the presence of rotting bulbs during harvest. These bulbs were discarded and not weighted. Supriyadi *et al.* (2013) reported that diseases can spread widely in shallot cultivation in both conventional and modern agriculture. The high intensity of *Fusarium* sp. attack on shallots reduces the productivity. According to Rahayu (2013) the reduction of shallot production can be caused by seasonal factors, especially during the peak of the rainy season. Too high rainfall will increase the attack of plant pathogen such as *Fusarium* sp., so that many bulbs decay when harvested.

Table 4. wet and dry weight of shallots

Treatments		Wet Weight (kg/treatment)	Dry Weight (kg/treatment)
Control Chemical Fungicide	Tajuk	3.00 ± 1.44	2.10 ± 0.82
	Biru Lanchor	2.40 ± 0.36	1.93 ± 0.31
	Super Philip	2.77 ± 0.87	1.73 ± 0.50
Concentration 5 ml/liter	Tajuk	1.93 ± 0.25	1.40 ± 0.26
	Biru Lanchor	1.70 ± 0.44	1.17 ± 0.40
	Super Philip	2.30 ± 0.56	1.37 ± 0.42
Concentration 10 ml/liter	Tajuk	2.23 ± 0.12	1.70 ± 0.17
	Biru Lanchor	2.43 ± 0.75	1.57 ± 0.55
	Super Philip	2.10 ± 0.30	1.20 ± 0.10

CONCLUSION

The conclusions from this study were: (1) Fobio biopesticide concentration of 10 ml/liter suppressed moler disease incidence on Tajuk, Super Philip, and Biru Lanchor shallot cultivars with the disease incidence of 18.78% at 42 DAP, the same as the application of chemical fungicide. (2) Fobio biopesticide concentration of 10 ml/liter had the same effect as chemical fungicide on the production of various shallot cultivars tested, with the wet weight of 2.25 kg/treatment and a dry weight of 1.49 kg/treatment.

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