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Propagation and shelf-life of weed pathogenic fungi in alternative media and their effectiveness in billygoat (*Ageratum conyzoides* L)

Perbanyakan dan daya simpan jamur patogen gulma pada media alternatif, serta efektivitasnya pada babandotan (Ageratum conyzoides L)

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Abstrak

*Pengendalian babandotan (*Ageratum conyzoides *L) saat ini dilakukan menggunakan herbisida, di lain pihak pengendalian menggunakan jamur pathogen bersifat ramah lingkungan, namun untuk perbanyakan massal dan penyimpanannya diperlukan media alternatif. Tujuan penelitian adalah untuk mengetahui jenis media alternatif terbaik untuk pertumbuhan dan umur simpan jamur, serta efektivitasnya terhadap babandotan. Perlakuan yang diuji merupakan kombinasi dua faktor yaitu jenis jamur pathogen (*Culvularia lunata *atau* Fusarium oxysporum*) dan jenis media (air cucian beras atau limbah cair tahu). Rancangan acak lengkap digunakan untuk uji in vitro sedangkan uji in planta menggunakan rancangan acak kelompok dan masing-masing unit percobaan diulang lima kali. Peubah yang diamati adalah kerapatan konidia, jumlah koloni, masa inkubasi, gejala penyakit, intensitas penyakit, area under the disease progress curve (AUDPC), tinggi, jumlah daun, bobot segar dan kering tajuk serta akar gulma. Hasil penelitian menunjukkan bahwa kerapatan konidium* F. oxysporum *57% lebih tinggi pada air cucian beras dibandingkan limbah cair tahu. Umur simpan terbaik untuk jamur adalah empat minggu. Penggunaan air cucian beras untuk* F. oxysporum *dan* C. lunata *masing-masing efektif menunda masa inkubasi 77 dan 71 %, menekan intensitas penyakit 90 dan 88 %, dan AUDPC 94 dan 93% dibandingkan kontrol. Jamur* F. oxysporum *yang ditumbuhkan pada medium air cucian beras mampu menurunkan jumlah daun, bobot segar, dan bobot kering babandotan masing-masing sebesar 25, 30, dan 20 % dibandingkan kontrol.*

[Kata kunci: pengendalian hayati, gulma daun lebar, perbanyakan jamur, air cucian beras, penyimpanan,].

Abstract

Control of billygoat (*Ageratum conyzoides* L) currently uses herbicides, on the other hand, control using pathogenic fungi is environmentally friendly, but for mass propagation and storage, alternative media are needed. The aim of the study was to determine the best type of alternative media for fungal growth and shelf life, as well as its effectiveness against billygoat. The treatment tested involves a combination of two factors: the type of pathogenic fungi (*Curvularia lunata* or *Fusarium oxysporum*) and the type of medium (rice washing water or tofu liquid waste). A completely randomized design was used for the in vitro test, while in planta test used a randomized block design with each experimental unit repeated five times. The observed variables were conidia density, number of colonies, incubation period, disease symptoms, disease intensity, and area under the disease progress curve (AUDPC), as well as weed height, number of leaves, fresh and dry shoot, and root weights. The results showed that the conidia density of *F. oxysporum* was 57% better in rice washing water than in tofu wastewater. The best shelf life for the fungus was four weeks. The use of rice washing water for *F. oxysporum* and *C. lunata* effectively delayed the incubation period by 77 and 71% respectively, suppressed disease intensity by 90 and 88%, and AUDPC by 94 and 93% compared to the control. The *F. oxysporum* grown on rice washing water media was able to reduce the number of leaves, fresh and dry weight of billygoat by 25, 30, and 20% compared to the control, respectively.

[Key words: biological control, broad leaves weed, fungal propagation, rice washing water, preservation].

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Introduction

Weeds especially billygoat (*Ageratum conyzoides* L) are plant-disturbing organisms causing yield loss and high costs for their management (Gharde et al., 2018; Koç et al., 2019). Negi et al. (2020) reported that billygoat is the dominant weed in various crop cultivation, especially plantation crops. The loss is caused by several factors such as competition for nutrients, sunlight, water, and living space (Gharde et al., 2018), as well as the influence of allelochemical compounds released by weeds and disrupting plant cell division, seed germination, and physiology (Shirgapure & Ghosh, 2020). Many billygoat weed management has been carried out, either manually, mechanically, or chemically using herbicides. Manual weed management is ineffective when applied to large areas, while mechanical weed management requires a lot of money (Loddo et al., 2021). Herbicides are widely applied to manage weeds because of their practicality, time saving, and ease of application (Singh et al., 2020), but the negative impacts of using herbicides occur in the agricultural world, especially in post-harvest products (van Bruggen et al., 2021).

Biological weed control is seen as safer and more environmentally beneficial. The biological control agents generally used are fungi, because of their destructive properties, mass production, direct formulation, and direct applications (Hyde et al., 2019). One of the biological control fungi for weeds is *Fusarium oxysporum* (Soesanto et al., 2020; Asim et al., 2022) and *Curcularia lunata* (Li et al., 2013). Both fungi have received special attention in biological weed management. The *F. oxysporum* has been used to control broadleaf weeds, while *C. lunata* was developed as a mycherbicide to control barnyardgrass (*Echinochloa crus-galli*).

Fungal biological agents need to be propagated using certain media before being applied in the field. Media for fungal propagation are a material consisting of a mixture of nutrients (Velivelli et al., 2014). The media often used for fungal propagation is laboratory media, but this media for mass propagation requires high costs (Uthayasooriyan et al., 2016; Soesanto et al., 2021). Therefore, the propagation of biological agents requires alternative means of propagation which are cheap and easy to obtain. Various carbohydrate and protein sources have been successfully used as alternative media for microbial growth (Ravimannan et al., 2014; Uthayasooriyan et al., 2016). Some organic wastes can be used as alternative media, such as rice washing water and tofu liquid waste, which are used as media for the propagation of biological agents including fungi (Asiandu et al., 2021; Afifah et al., 2022). Rice washing water is an organic waste that contains high levels of carbohydrates, protein, gluten, and vitamins. In addition, rice washing water contains vitamins such as niacin, riboflavin, and thiamin, as well as minerals such as Ca, Mg, and Fe which are needed for fungal growth (Abba et al., 2021). The rice washing water has been found to contain several essential plant nutrients such as (in mg L^{-1}) 40 to 150 of N, 4.19 to 10.14 NO_3^--N, 2.57 to 39.72 NH_4^+-N, 43 to 1630 P, 51 to 200 K, 8 to 2944 Ca, 36 to 1425 Mg, and 27 to 212 S (Nabayi et al., 2022). Another medium for microbial multiplication is tofu liquid waste. Organic components such as carbohydrates, fats, and nitrogen contained in tofu liquid waste can accelerate the growth of mycelia and optimum fungal production (Wang et al., 2018; Li et al., 2021). Both of these alternative media should be tested for the propagation and storage of weed-pathogenic fungi. The purpose of this study was to determine the suitable alternative media type to support the growth and shelf life of pathogenic fungi and the effectiveness of weed pathogenic fungi to control billygoat weed.

Materials and Methods

This research was conducted at the Plant Protection Laboratory and in the greenhouse, Faculty of Agriculture, Jenderal Soedirman University, from October 2021 to February 2022.

Preparation of weed-pathogenic fungi

The weed pathogenic fungi used were *F. oxysporum* and *C. lunata*. Every weed pathogenic fungi was inoculated as much as one cork drill (6 mm diameter) into the center of the PDA in a separate Petri dish. The culture was then incubated for 7 days or until the fungi filled the Petri dish (Soesanto et al., 2021).

Preparation of liquid waste

The rice washing water medium was prepared by collecting 800 mL of rice washing water plus 200 mL of coconut water and 15 g L^{-1} of sugar and boiled (Awasthi et al., 2011). After boiling, the solution was measured for its pH reaching a pH of 6.5-7.0, then filtered and put directly into a 250 mL Erlenmeyer flask. Furthermore, the rice washing water was sterilized by autoclaving at 121 ^{O}C for 30 min. Tofu liquid waste media was made by utilizing as much as 1500 mL tofu liquid waste from the tofu factory, filtering using cheesecloth, then the pH of the tofu liquid waste was adjusted by adding 10% NaOH solution to pH 6.5-7.0 (Purnama et al., 2012). Furthermore, tofu liquid waste was sterilized using an autoclave at 121 ^{O}C for 30 min.

Propagation and storage of weed pathogenic fungi

Five pieces of each pathogenic fungal isolate (6 mm in diameter) were put into an Erlenmeyer containing liquid waste media, then shaken for 7 days using a shaker at 150 rpm at room temperature (± 28 ^{O}C). *F. oxysporum* and *C. lunata* which had been shaken were then stored for 10 weeks. Conidia density calculations were carried out every 2 weeks for 10 weeks.

Preparation of billygoat

Billygoat is prepared from seeds harvested from old weeds. Seeds were sown on fine sand media in plastic trays, watered daily as needed, and then placed in the shade. The billygoat seedlings used in this study were those with 4 leaves. Weeds were planted in a simple cultivated field with an area per plot of 1.5 × 1.5 m^2, with a 25 × 25 cm^2 spacing (Scholz et al., 2014).

Application of the weed pathogenic fungi to control billygoat

Weed samples aged 14 days after planting (DAP) were sprayed with spore or microconidia density of 3.31×10^7 spore mL^{-1} for *C. lunata* suspension and 37.45×10^7 microconidia mL^{-1} for *F. oxysporum* that had been stored for 8 weeks. Pathogenic fungi *F. oxysporum* and *C. lunata* in each alternative media were applied by spraying using knapsack sprayer five times on the underside of leaves with an interval of three days in the afternoon.

Experimental design

The experimental design used in the laboratory was a completely randomized design factorial, consisting of two factors, namely weed pathogenic fungi (*C. lunata* and *F. oxysporum*) and the type of media (rice washing water and tofu liquid waste) with 4 repetitions. The experimental design used in the greenhouse was a randomized block design consisting of a control; rice washing water + *F. oxysporum*; rice washing water + *C. lunata*; tofu liquid waste + *F. oxysporum*; and tofu liquid waste + *C. lunata*, with five replicates. In total there were 25 experimental units and each experimental unit had 3 weeds.

Observed variables

The conidia density of *C. lunata* and *F. oxysporum* can be calculated with a hemocytometer using the formula (Walter et al., 2010), namely: density of conidia per mL solution resulted from the average number of conidia in boxes a, b, c, d, and e divided by the volume of the sample box. The incubation period was calculated from inoculation until the first symptoms appeared in units of days after inoculation (dai) (Leclerc et al., 2014). Observation of the intensity of the disease was carried out at intervals of 3 days. Disease intensity can be calculated by the formula (Bhat et al., 2013):

$$DI = \frac{\sum(nxv)}{N\ X\ Z} \text{ x } 100\%,$$

where DI = disease intensity; v = Value (score) of disease intensity based on leaf area of all symptomatic plants; n = number of plants with the same v value; Z = highest value (score) (v=5); N = number of plants observed. The score used was 0 = disease free, 1 = infected leaf area 0.1-10%, 2 = infected leaf area 10.1-25%, 3 = infected leaf area 25.1-50%, 4 = leaf area infected 50.1-75%, and 5 = leaf area infected >75.1%. AUDPC was calculated using the integration of the trapezoid method (Paraschivu et al., 2013), with the following formula:

$$AUDPC = \sum_{i}^{n-1} \left|\left(\frac{Y_{i+1} + Y_i}{2}\right)\right| t_{i+1} - t_i$$

where: Y_i = disease severity on the ith date; t_i = ith day; n = number of dates on which the disease was recorded.

The height of the first weed was measured from the base of the root to the tip of the highest leaf when it was cupped using a ruler (cm) (Gfeller et al., 2018). The number of leaves was counted for each weed after the first application. Fresh shoot weight was measured by separating weed shoot from roots, cleaned of dirt, weighed using an analytical balance, and dried at 60 °C using an oven for 2 x 24 hours to obtain dry shoot weight.

Data analysis

Data were analyzed by the F test at 5% error rate. If the results of the analysis show that there is a significant effect of the treatment, then a further test is carried out using the Duncan Multiple Range Test (DMRT) at an error level of 5%.

Result and Discussion

Density of weed pathogenic fungi

The conidial density of *F. oxysporum* was higher than *C. lunata*. The treatment of pathogenic fungal species and media types showed significant differences (Table 1). Rice-washing water is better medium for the propagation of pathogenic fungi compared to tofu liquid waste media. *F. oxysporum* is capable of producing a microconidia density of 1.313 x 10^8 microconidia mL^{-1} or increasing 75 % than *C. lunata* in the rice washing water medium.

Table 1. The density of conidia of pathogenic fungi after 10 weeks of storage
Tabel 1. Kepadatan konidium jamur patogen setelah penyimpanan 10 minggu

Treatments *Perlakuan*	Conidia density ($\times 10^7$ conidia mL^{-1}) *Kepadatan konidium ($\times 10^7$ konidium mL^{-1})*
Growth media	
Rice washing water	17 x $10^{7\ a*)}$
Tofu liquid waste	7 x $10^{6\ b}$
Pathogenic fungi	
C. lunata	3.31 [a]
F. oxysporum	37.45 [b]
Combination of growth media x pathogenic fungi	
C. lunata x rice washing water	3.31 [a]
C. lunata x tofu liquid waste	3.13 [bc]
F. oxysporum x rice washing water	13.13 [d]
F. oxysporum x tofu liquid waste	8.55 [c]

[*)]Note: Numbers followed by different letters in the same row indicated significantly different according to DMRT with a level of 5%.
[*]*Keterangan: Angka diikuti huruf berbeda pada baris yang sama menunjukkan tidak berbeda nyata menurut DMRT pada taraf 5%.*

Even the rice-washing water media produced a better density of fungal conidia, which was 57 % compared to tofu liquid waste media. This is because the rice-washing water media contains lots of nutrients that can promote fungal growth (Abba et al., 2021). The addition of coconut water to the rice washing water medium will induce fungal cells to divide and grow rapidly (Magday et al., 2014). The *F. oxysporum* is able to use carbon or carbohydrate sources contained in rice washing water media and also tofu liquid waste (Ali et al., 2018). Each fungus has a different ability to use carbon sources around it (Lok et al., 2021). Based on pathogenic fungi, *F. oxysporum* is the most suitable pathogenic fungus to grow on both types of liquid media compared to the other pathogenic fungi. This is because *F. oxysporum*, which is a soil-borne fungus, has the ability to degrade organic waste with the enzymes it produces, so that it can utilize both of these wastes compared to the pathogenic fungi (Steinkellner et al., 2005; Anasontzis & Christakopoulos, 2014).

Shelf life of weed pathogenic fungi

Based on Figure 1, it can be seen that the lag phase of *C. lunata* in both media occurred in the 1st to 2nd week of storage. The log phase occurs from the 2nd to the 4th week of storage. This is because the fungus uses the nutrients in the media to grow and develop. The stationary phase occurs at the beginning of the 4th week, where fungal growth is relatively steady and reaches the highest number of conidia; then from the 4th week of storage there was a decrease until the 10th week. This decrease occurs due to limited nutrient content in the media (Nottingham et al., 2018). *Curvularia* sp. grows better and faster on both organic media types than other pathogenic fungi. Organic liquid waste has good potential as a medium for fungal propagation (Alibardi et al., 2020). In addition, toxic compounds are also formed which inhibit and kill the fungal conidia (Rukmini et al., 2017; Ebadzadsahrai et al., 2020).

The density of conidia *C. lunata* in the rice washing water medium was higher than in the tofu liquid waste media. This indicates that the nutrient content in the rice washing water medium added with coconut water is more suitable for the growth of *C. lunata* in tofu liquid waste (Abba et al., 2021). The highest population was seen in the 4th week of observation, and the lowest population was seen in the 10th week. The addition of coconut water to rice washing water media will accelerate the growth of fungal cells (Magday et al., 2014).

The lag phase of *F. oxysporum* in both media occurred from the 1st to the 2nd week of storage (Figure 2). The log phase in the medium of rice washing water and tofu liquid waste starts from the 2nd week until the 4th week of storage, which is consistent with *C. lunata* (Figure 1). Like every living thing, fungi need energy and food sources to complete their development. Macro- and micronutrients are essential for fungal growth and reproduction. Fungi need vitamins for their growth and development, such as thiamine, biotin, riboflavin, nicotinic acid, vitamin K, and pantothenic acid (Wongjiratthiti & Yottakot, 2017). The microconidia density increased higher in the rice washing water medium than in the tofu liquid waste media. This is due to the media nutrient content for the growth of *F. oxysporum*. Rice-washing water media contains higher carbohydrates and other essential minerals than tofu liquid waste media (Abba et al., 2021; Nabayi et al., 2022). Carbohydrates are a source of nutrition in fungal culture media (Wongjiratthiti & Yottakot, 2017). The 10th week of observation showed that *F. oxysporum* began to enter the death phase (Figure 2). The stationary phase is seen in the 4th to 8th weeks of storage.

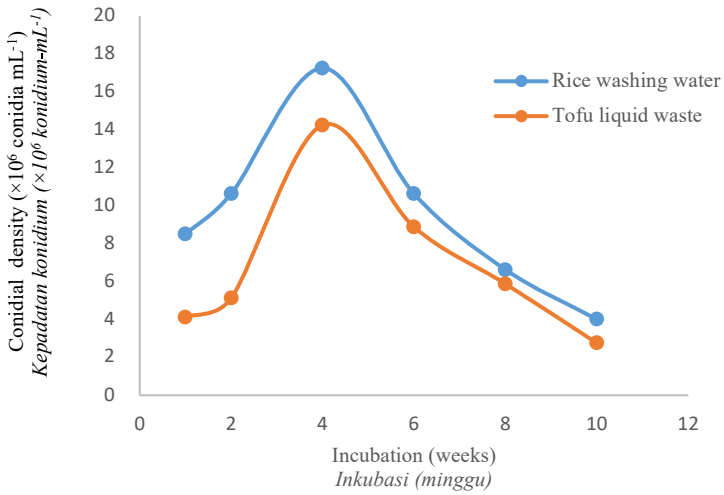

Figure 1. Conidial density of *C. lunata* on rice washing water and tofu liquid waste media.
Gambar 1. Kepadatan konidium C. lunata pada medium air cucian beras dan limbah cair tahu.

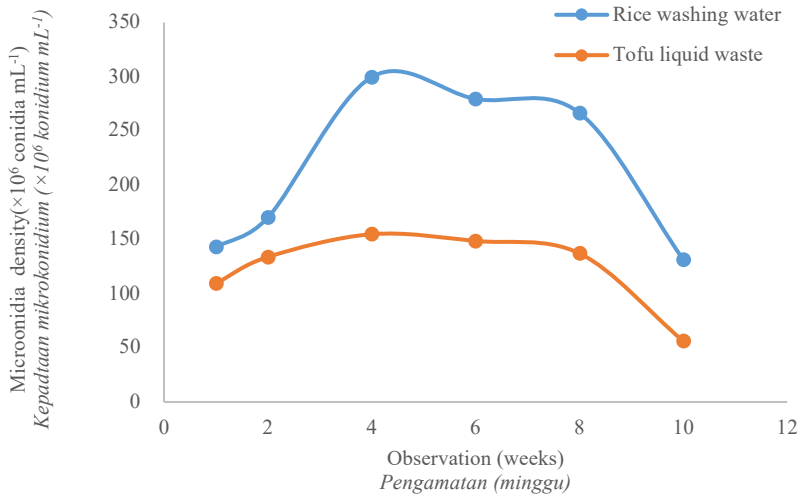

Figure 2. Microconidia density of *F. oxysporum* in rice washing water and tofu liquid waste media.
Gambar 2. Kepadatan mikrokonidium F. oxysporum dalam medium air cucian beras dan limbah cair tahu.

Effect of the treatments on pathosystem components

Application of *C. lunata* causes symptoms of brownish leaf spots, the longer the spots get bigger, after that the billygoat weed leaves begin to appear translucent (Figure 3). This is in accordance with the opinion Park et al. (2020) that the early symptoms of leaf spot disease due to *C. lunata* in the form of small round spots, translucent yellow in color that infect the crown and leaf blade, which over time become dry, gray-brown spots, so they shrink and die.

Symptoms that appear on billygoat weed infected with *F. oxysporum* are leaves that appear to curl, leaf color changes from fresh green to yellow to brown, necrosis, and then dried (Figure 4). The initial symptoms of *Fusarium* sp. are characterized by symptoms of chlorosis on the leaf blade, then a red line will appear (Joshi, 2018).

Figure 3. The attack of *C. lunata* on billygoat leaves. (a) initial symptoms of brown spotting, (b) translucent yellow leaves, and (c) gray-brown dry spots, shriveled and dead.
Gambar 3. Serangan C. lunata pada daun babandotan. (a) gejala awal noda coklat, (b) daun daun berwarna kuning bening, dan (c) bintik kering berwarna abu-abu kecokelatan, layu dan mati.

Figure 4. *F. oxysporum* attack on billygoat leaves. (a) leaf curl, (b) necrosis, and (c) wither and die.
Gambar 4. Serangan F. oxysporum pada daun billygoat.(a) daun menggulung, (b) nekrosis, dan (c) layu dan mati.

The shortest incubation period for two pathogenic fungi in liquid organic media was the application of *F. oxysporum* + rice washing water, which was 4 days after inoculation or could accelerate the incubation period by 78 % compared to control (Table 2). Application of rice washing water medium and *F. oxysporum* or *C. lunata* with the conidia density of 10^6 conidia mL^{-1} each, accelerate the incubation period of billygoat better than in tofu liquid waste medium. It is suspected that the two weed pathogenic fungi are able to multiply in the rice washing water medium because they are able to utilize the nutrients in it, so that the fungi can attack more weeds. This condition is in accordance with the statement that the rice washing water medium contains several nutrients needed for the growth of pathogenic fungi (Abba et al., 2021). The amount of propagules of weed pathogenic fungi determines the level of weed disease infection and will determine the duration of the incubation period (Doehlemann et al., 2017).

Table 2. Pathosystem components in the treatment of weed pathogenic fungi + alternative media
Tabel 2. Komponen patosistem dalam perlakuan jamur patogen gulma + medium alternatif

Treatments *Perlakuan*	Incubation period (dai) *Masa inkubasi (hsi)*	Disease intensity (%) *Intensitas penyakit (%)*	AUDPC (% days) *AUDPC (% hari)*
Control	18.50 $^{e*)}$	4.00 ^{a}	20.14 ^{a}
F. oxysporum + rice washing water	4.00 ^{a}	45.52 ^{e}	351.47 ^{g}
C. lunata + rice washing water	5.30 ^{b}	34.94 ^{d}	291.78 ^{f}
F. oxysporum + tofu liquid waste	7.61 ^{d}	33.20 ^{cd}	247.21 ^{e}
C. lunata + tofu liquid waste	6.66 ^{cd}	29.63 ^{b}	233.00 ^{cde}

$^{*)}$ Note: Numbers followed by different letters in the same column indicated significantly different according to DMRT with a level of 5%. dai = days after inoculation.
$^{*)}$*Keterangan: Angka diikuti huruf berbeda pada kolom sama menunjukkan berbeda nyata menurut DMRT pada taraf 5%. hsi = hari setelah inokulasi*.

The faster incubation period in the application of *F. oxysporum* + rice washing water also determines higher disease infections (Table 3). *F. oxysporum* is able to penetrate directly into the cuticle layer with the help of cell wall decomposing enzymes which play a role in pathogenesis resulting in cell wall damage (Gonzalez-Roncero et al., 2000). Polygalacturonase secretion is one of the key conditions for infection in plants (Deising et al., 1995). Meanwhile, *C. lunata* has the ability to synthesize proteins identified as related to the process of virulence differentiation (Gao et al., 2014).

Based on AUDPC data, the application of *F. oxysporum* + rice washing water medium has a higher AUDPC value than other treatments (Table 2 & Figure 5). This condition is consistent with the high intensity of the disease and the short incubation period. The high intensity of the disease in a unit of time will affect the AUDPC value (Ramirez-Gil et al., 2017). *F. oxysporum* which was propagated in rice washing water medium was able to infect billygoat faster and the disease intensity was higher than other treatments. The higher the AUDPC value, the more effective the pathogen is in controlling billygoat weed (Cohen et al., 2018).

Effect of the treatments on growth components

Application of *F. oxysporum* and *C. lunata* in organic liquid waste media has not a significant effect on weed height (Table 3). This is due to the relatively low intensity of the disease due to weed pathogenic fungi, so it has not affected the weed height. In accordance with the opinion that low disease intensity does not affect plant morphology (van Dijk et al., 2021). In the data on the number of leaves, the application of the two weed pathogenic fungi reduced significantly compared to control. The two weed pathogens, especially *F. oxysporum*, showed a decrease in the number of leaves by 25 and 31% in rice washing water and tofu liquid waste media, respectively; while *C. lunata* in tofu liquid waste reduced the number of leaves by 60%. However, the largest decrease in the number of leaves due to *C. lunata* was not followed by high pathosystem component (Table 2). *F. oxysporum* is a fungal pathogen that attacks the leaf veins by blocking the flow of nutrients and water from the soil, thereby reducing the supply of photosynthesis and inhibiting the emergence of new leaves (Joshi, 2018).

The canopy fresh weight of babandotan treated by the two weed pathogenic fungi in organic wastewater was lower than those of control. The application of weed pathogenic fungi in liquid organic waste can reduce the fresh and dry weight of leaves (Table 3). The *F. oxysporum* in rice washing water media reduced leaf fresh weight by 30% while *C. lunata* in tofu liquid waste media reduced leaf fresh weight by 35% each compared to control. Organic liquid waste supports the multiplication of weed pathogenic fungi (Neher et al., 2013; Omeike et al., 2019), thereby increasing the number of conidia per mL, pathosystem components, and reducing weed growth. The fresh weight of weeds was lower due to the attack of weed pathogenic fungi. This is consistent with the number of leaves decreased.

The application of weed pathogenic fungi + alternative media has a significant effect on weed dry weight (Table 4). The low dry weight of weeds is consistent with the low fresh weight of weeds, which is caused by the application of weed pathogenic fungi in liquid organic waste media. The *F. oxysporum* in rice washing water reduced weed dry weight by 20% compared to control, while all the treatments were not significantly different. The low dry weight of weeds is also caused directly by the low fresh weight of weeds (Huang et al., 2019). The low weed mass supported by the low number of leaves will affect the dry weight of the weeds. This condition proves that the low growth of weeds is caused by the application of *F. oxysporum* which is propagated in rice washing water media (Steinkellner et al., 2005; Joshi, 2018).

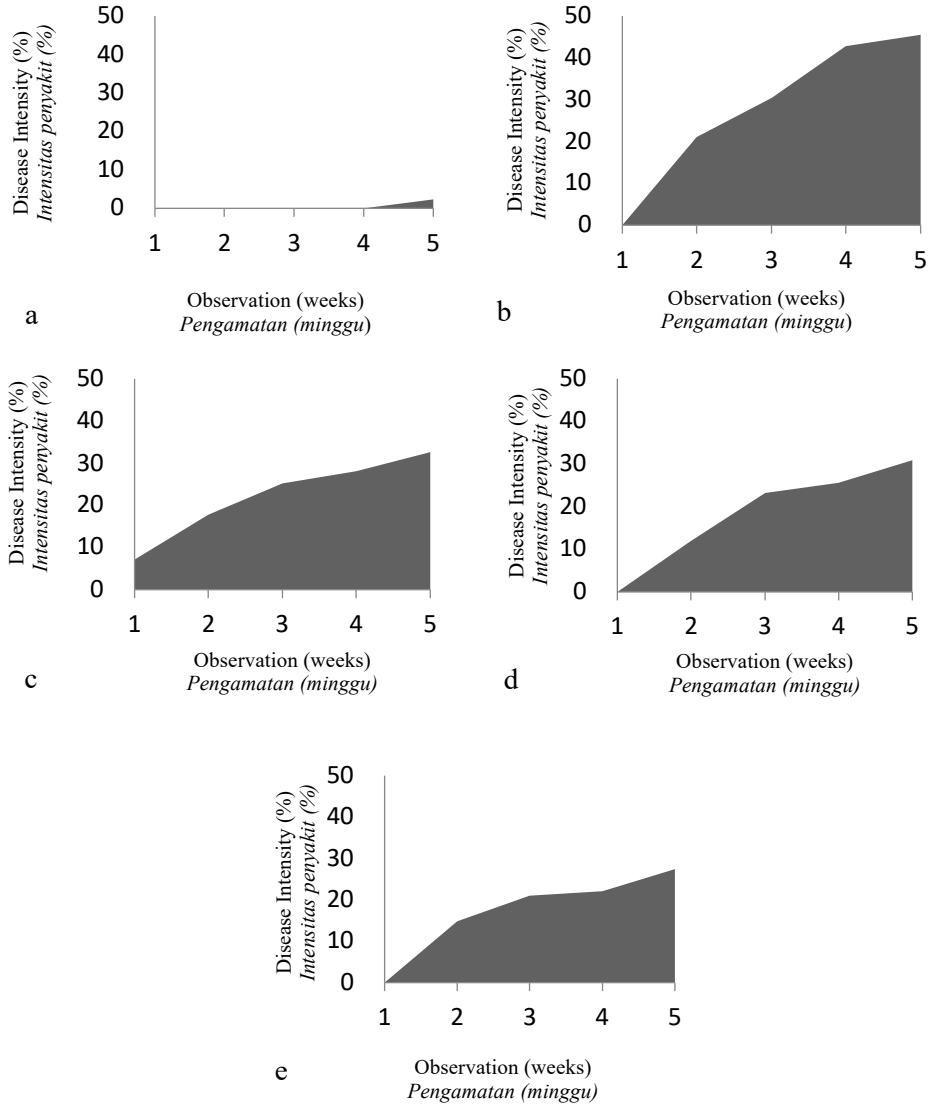

Figure 5. AUDPC in organic liquid waste media. a) Control, b) *F. oxysporum* + rice washing water, c) *C. lunata* + rice washing water, d). *F. oxysporum* + tofu liquid waste, and e) *C. lunata* + tofu liquid waste.
Gambar 5. AUDPC dalam medium limbah cair organic. a) Kontrol, b) F. oxysporum + *air cucian beras, c)* C. lunata + *air cucian beras, d)* F. oxysporum + *limbah cair tahu, dan e)* C. lunata + *limbah cair tahu*.

Table 3. Components of weed growth in the treatment of weed pathogenic fungi + liquid organic media
Tabel 3. Komponen pertumbuhan gulma pada perlakuan jamur patogen gulma + medium organik cair

Treatments *Perlakuan*	Weed height *Tinggi gulma*	Number of leaves *Jumlah daun*	Fresh weight of canopy *Bobot segar kanopi*	Dry weight of canopy *Bobot kering kanopi*
Control	3.25 [a*)]	28.00 [c]	13.90 [b]	1.78 [b]
F. oxysporum + rice washing water	3.69 [a]	20.90 [b]	9.73 [a]	1.43 [a]
C. lunata + rice washing water	3.42 [a]	20.69 [b]	10.33 [a]	1.41 [a]
F. oxysporum + tofu liquid waste	3.27 [a]	19.21 [b]	11.72 [a]	1.51 [a]
C. lunata + tofu liquid waste	2.94 [b]	11.22 [a]	9.00 [a]	1.33 [a]

*) Note: Numbers followed by different letters in the same column indicated significantly different according to DMRT with a level of 5%.
*)*Keterangan: Angka diikuti huruf berbeda pada kolom sama menunjukkan berbeda nyata menurut DMRT pada taraf 5%.*

Conclusion

The best alternative medium for the growth of pathogenic fungi is rice washing water with a percentage of 57 % compared to tofu liquid waste. The optimum shelf life of *C. lunata* and the best *F. oxysporum were* 4 weeks. Application of *F. oxysporum* or *C. lunata* in rice washing water media was able to cause damage to billygoat weed leaves respectively seen from reduction of the incubation period of 77 and 71%, disease intensity of 90 and 88%, and AUDPC 94 and 93% compared to control. *F. oxysporum* in rice washing water media reduced the number of leaves, fresh and dry weight of weeds by 25, 30, and 20%, respectively, compared to control.

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