

The use of alternative liquid media for propagation of pathogenic fungi and their effect on weeds

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Abstract. Soesanto L, Mugiastuti E, Manan A. 2021. *The use of alternative liquid media for propagation of pathogenic fungi and their effect on weeds.* Biodiversitas 22: 719-725. Weeds cause high yield losses and are controlled by chemical, physical and mechanical control. Weed pathogenic fungi could be used as a biological control agent. However, the propagation media of them are still a higher cost, therefore, the new alternative and inexpensive media for weed pathogenic fungi propagation are required. The propagation of weed pathogenic fungi in liquid media in-vitro used randomized completely design consisted of three pathogenic weed fungi, i.e., *Chaetomium* sp., *Fusarium* sp., and *Curvularia lunata* and three liquid media, i.e., PDB, rice washing water, and tofu liquid waste. The in-plant tests used split-plot design used weed pathogenic fungi multiplied in the best formula from in-vitro results (rice washing water) and control as the main plot and four weeds, i.e., *Cyperus kyllingia*, *Cynodon dactylon*, *Portulaca oleracea*, and *Ageratum conyzoides* as subplot. The variables observed were conidia density, incubation period, disease intensity, number of leaves, and weed height. The results showed that rice washing water and tofu liquid waste were suitable media for the propagation of the weed pathogenic fungi. *Chaetomium* sp. was virulent fungi against broad leaves weeds, especially *A. conyzoides*. This fungus can be promoted to manufacture organic herbicides against the weed.

Keywords: Alternative liquid media, fungal propagation, weed pathogenic fungi

INTRODUCTION

Weeds are the largest pest problem in Agriculture causing yield losses as well as cost management control. Agricultural weeds have emerged rapidly competing with crops for nutrients and water. They also produce chemicals that suppress plant growth and act as alternative hosts for plant pests and pathogens. Their seeds can germinate annually leading to light competition with crop plants, increase fluctuations in soil temperature and humidity, and accelerate the release of nutrients (Kilkoda et al. 2015; Cai and Gu 2016). The losses caused by weeds in crops vary depending on the type of product cultivated such as peanuts (35.8%), soybeans (31.4%), corn (25.3%), sorghum (25.1%), wheat (18.6%), and rice (Gharde et al. 2018).

Currently, weed control has been carried out by chemical (herbicide), physical and mechanical control. Physical-mechanical control requires a lot of labor, time-consuming, and money (Cai and Gu 2016). Controlling using herbicides intensively and less wisely can have negative effects, including toxicity to main crops, weed resistance, environmental contamination, negative effects on non-target organisms and soil, water and food pollution (Mada et al. 2013; Van Bruggen et al. 2017). Herbicide granules that fall to the ground within a certain period will change and be absorbed in the clay fraction and soil organic matter (Tabroni et al. 2018). Therefore, it is necessary to look for other environmentally-friendly control alternatives.

Biological control is an alternative to reduce weed populations in plants. Weed biological control is developed

using organisms, such as insects, nematodes, bacteria, fungi, or live natural products (Schwarzländer et al. 2018). According to Fauzi et al. (2012) and Harding and Raizada (2015), fungi are the most studied and used pathogens. Fungi are very common in plants, have destructive properties, produce large quantities, and be formulated and commercialized.

The propagation of pathogenic fungi is a very important step in biological control using pathogenic fungi. Up to now, the media frequently used for fungi propagation is potato dextrose broth (PDB) media (Agale et al. 2018; Venkatachalam et al. 2019); although the medium for mass propagation requires a higher cost. Therefore, a new alternative medium can be used as a culture medium with low economic value, adequate nutrition, effectiveness, obtaining abundant raw material availability, and fungi can use that to grow and develop.

Some organic wastes can be used as an alternative media to propagate weed pathogenic fungi (Karimi et al. 2018). Rice washing water has the potential to substitute potatoes in pure fungal culture media because the rice washing water contains high carbohydrates, gluten, protein, and vitamins (Chethana et al. 2011). Tofu liquid waste can also be used for the growth of various microbes because it contains many nutrients such as nitrate, phosphate, and ammonium (Juariah 2018). The use of liquid tofu waste medium is very economical in terms of cost. Based on this, research on the propagation of weed pathogenic fungi with an alternative liquid medium of washing rice and tofu liquid waste and its effect on weeds needs to be carried out.

This study aimed to determine the effect of rice washing water and tofu liquid waste on developing pathogenic fungi and their effects on several tested weeds.

MATERIALS AND METHODS

Preparation of liquid media

Liquid media of rice washing water was prepared by washing rice with water (1: 2, w/v) modified from Awasthi et al. (2011). Tofu liquid waste media was obtained from the tofu industry and prepared using Purnama et al. (2012).

Preparation of weed pathogenic fungal isolates

Weed pathogenic fungi, *i.e.*, *Chaetomium sp.*, *Fusarium sp.*, and *Curvularia lunata*, were prepared in PDA (Soesanto et al. 2020). Furthermore, the three weed pathogenic fungi were propagated using three liquid media, *i.e.*, Potato Dextrose Broth (PDB), rice washing water, and tofu liquid waste, which were shaken out at 150 rpm (Daiki) at room temperature for 7 days.

Preparation of growing media

The weed growing media was prepared by mixing the soil and manure, with a ratio of 2: 1. Then the media was put into a polybag with the size of 3 kg of media.

Preparation of weeds

The weeds that are used are prepared through weed seeds and sown on sterile filter paper and moist until they come out of the roots, then transferred to polybags, so that a homogeneous weed material is obtained.

In vitro test

Into the cold sterile liquid media, three pieces of pathogenic fungal isolates were inserted, which were cut with a 6 mm diameter sterile cork borer and incubated for 7 days (Fauzi et al. 2012). *In-vitro* testing of weed pathogenic fungi on the liquid media used a factorial randomized block design repeated three times. The first factor was three isolates of fungal pathogens, including *Chaetomium sp.*, *Fusarium sp.*, and *C. lunata*. The second factor was three types of liquid media consisted of PDB as a control, rice washing water, and tofu liquid waste.

In planta test

The in planta test used a split-plot design with three replicates. The main plot consisted of fungal pathogens with the best formula resulting from in vitro test (rice washing water), namely without pathogen inoculation as a control, *Chaetomium sp.*, *Fusarium sp.*, and *C. lunata*. The subplots consisted of four species of weeds that are often found around the plantations, *i.e.*, *Cyperus kyllingia*, *Cynodon dactylon*, *Portulaca oleracea*, and *Ageratum conyzoides*. In planta, test was carried out by spraying a 7-day-old suspension of pathogenic fungi with a conidia density of 1×10^6 conidia/mL against test weeds 7 days after planting (dap), which had been grown on polybags. Spraying was carried out on the underside of the leaves and repeated 4 times with 7-days intervals (Fauzi & Murdan 2009).

Observed variables

The variables observed in the *in vitro* test included conidia density of weed pathogenic fungi, which was observed every two days, for eight days after inoculation (Novianti 2018). The variables observed in the *in vivo* test include incubation period (observed from inoculation until the onset of symptoms (Wiyatiningsih et al. 2009)); disease intensity, AUDPC (Area Under the Disease Progress Curve), plant height, and the number of leaves (observed every 7 days).

The disease intensity is calculated every 7 days by the formula (Gashaw et al. 2014):

$$DI = \frac{\sum(n \times v)}{N \times Z} \times 100\%$$

Where: DI = disease intensity (%), n = Number of plants in each category, v = Numerical values of symptoms category, N = Total number of plants, and Z = Maximum numerical value of symptom category. Numerical value used to assess the disease intensity by using 0 to 5 rating scale (Bhat et al. 2013), as follows 0 = disease free, 1 = 0.1-10% the leaves attacked, 2 = 10.1-25% the leaves attacked, 3 = 25.1-40% the leaves attacked, 4 = 50.1-75% the leaves attacked, and 5 = >75% the leaves attacked.

AUDPC was calculated by using formula (Paraschivu et al. 2013):

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

Where: Y_i = disease severity on the i th date; t_i = i th day; n = number of dates on which the disease was recorded

Data analysis

Data were analyzed by F test and if there was a variation between treatments, it was continued with the Duncan Multiple Range Test (DMRT) at a level of 5%. All analyses were carried out using SPSS version 25.

RESULTS AND DISCUSSION

Conidia density of weed pathogenic fungi on *in-vitro* liquid media

The results showed that the types of liquid media were not significantly different from PDB as control (Table 1). This means that an alternative liquid media can be used to replace the PDB. These results were caused by the different nutritional content in each media. PDB is media widely used for fungal growth to produce antibiotics (Margino 2008; Elfita et al. 2012). The same situation also happened in the rice washing water used for fungal growth (Thiruvengadam et al. 2018). The nutrient content of white rice washing water included 0.015% nitrogen, 16.306% phosphorus, 0.02% potassium, 2.944% calcium, 14.252% magnesium, 0.027% sulfur, 0.0427% iron, and 0.043% vitamin B1 (Wulandari et al. 2012). Meanwhile, Karimi et al. (2018) reported that the growth of filamentous mushroom cultivation can be carried out in the waste liquid medium of several fermented food products, such as tofu, tempeh, and miso.

Table 1. Observation of conidia density for 6 days (when it reaches the stationary phase)

Treatments	Conidia density (x 10 ⁶ conidia mL ⁻¹)
Propagation media	
PDB	6.45 a
Rice washing water	7.88 a
Tofu liquid waste	7.12 a
Weeds pathogenic fungi	
<i>Chaetomium</i> sp.	5.82 b
<i>Fusarium</i> sp.	13.40 c
<i>Curvularia lunata</i>	2.23 a
Combination of media types and weed pathogenic fungi	
PDB- <i>Chaetomium</i> sp.	14.05 d
PDB- <i>Fusarium</i> sp.	4.90 c
PDB- <i>Curvularia lunata</i>	0.40 a
Rice washing water- <i>Chaetomium</i> sp.	3.40 bc
Rice washing water - <i>Fusarium</i> sp.	18.70 e
Rice washing water - <i>Curvularia lunata</i>	1.55 ab
Tofu liquid waste - <i>Chaetomium</i> sp.	0.00 a
Tofu liquid waste - <i>Fusarium</i> sp.	16.60 de
Tofu liquid waste - <i>Curvularia lunata</i>	4.75 c

Note: The number followed by the same letter shows that it is not significantly different according to the 5% DMRT level

In Table 1 also appears that the types of weed pathogenic fungi have significantly different conidia densities. *Fusarium* sp. has more conidia density than the other two tested fungi. This means that *Fusarium* sp. and all fungi can grow and develop in the liquid media. The growth of each fungus is determined, among other circumstances, by the nutritional content in the growth media, in addition to the fungi's genetic factors and the growing environment. Nutrition plays an important role in fungal growth. Based on Abdel-Kader et al. (2014), the growth of fungal mycelia decreased significantly as the concentration of essential oils was increased, to achieve minimum fungal growth at the highest concentrations used. Differences influence fungal growth in temperature, pH, and incubation period (Abdel-Kader et al. 2014; Ali et al. 2017).

Meanwhile, *Fusarium* sp. has the best capability to grow and develop in the alternative liquid media, i.e., rice washing water and tofu liquid waste (Table 1). This is because the nutrient content in rice washing water can be used for fungal growth (Wulandari et al. 2012). Several studies have shown that rice washing water is a liquid media for fungal growth, for example, on *Beauveria bassiana*, *Paecilomyces fumosoroseus*, and *Verticillium lecanii* (Sahayaraj & Namasivayam, 2008), *Aspergillus niger* (Handarini et al. 2018), and *Saccharomyces cerevisiae* (Mujdalipah & Daughter, 2020). The liquid waste of tofu also showed conidia density in the pathogenic fungus *Fusarium* sp. high and significantly different from other pathogenic fungi. This is because tofu liquid waste contains nutrients that can be used for fungal growth, for

example, tofu liquid waste for the growth of *Aspergillus flavus* (Utami and Suprihadi 2018).

The combination of the media and the pathogenic fungi gave different results (Table 1). *Chaetomium* sp. shows the best growth on PDB; while other fungi show less growth. This is due to the nutritional composition of the PDB. The PDB contains nutrients from potato and dextrose, which is described in the research results published by Fogle et al. (2009); there is stated that *C. globosum* grown in PDA showed optimum growth and produced secondary metabolites chaetoglobosin C at neutral pH. Besides, fungal spacing is preferable in an acidic environment. This is supported by the results of the experiment in Pan et al. (2016), who show that the optimum antifungal activity could be obtained by using potato dextrose broth (PDB) as the basic culture media and fermentation for 4-8 days (initial pH: 7.5) in *C. globosum*.

Chaetomium sp. growth was influenced by the cultivation media (Figure 1). It can be seen in Figure 1 that the *Chaetomium* sp. shows better growth in PDB. Fungi experience a stationary phase at the beginning of growth and begin to grow on day 2 to peak on day 6, and then decline. Even though its growth decreased on day 8, *Chaetomium* sp. still gave the best conidia density compared to the other two test fungi. It is assumed that the nutrient content in the growing liquid media has decreased, thus affecting the growth of fungi. Fungi have shown changes in their growth rate when nutrients are available. In general, five phases of filamentous fungal growth can be distinguished, i.e., the lag phase, the first transition phase, the log phase, the second transition phase, and the stationary phase (Meletiadiis et al. 2001). The growth curve is smooth and is characterized by a long transition period. The optimum nutrient media provides adequate growth of pathogenic fungi and the best growth to allow the fungus to grow without boundaries and express all phenotypes.

Fusarium sp. growth looks better on tofu liquid waste and rice washing water compared to *Chaetomium* sp. (Figures 2 and 1). On the 6th day, *Chaetomium* sp. reached conidia optimum at 14.05 x 10⁶ conidia/mL; but on the 6th day, *Fusarium* sp. only achieved conidia optimum at 4.9 x 10⁶ conidia/mL. This means that *Fusarium* sp. slower and fewer conidia form. This condition is caused by fungal genetic factors and external factors such as the type of propagation media and the environment. The genetic diversity of fungi affects the optimum growth of fungi (De Ligne et al. 2019). Meletiadiis et al. (2001) and De Ligne et al. (2019) reported that several parameters are involved in the growth of filamentous fungi, such as preparation of the inoculum, incubation conditions (time and temperature), and the type of nutrient medium. When glucose is the first nutrient-depleted, the fungus moves from an exponential growth phase to a very short and almost negligible stationary phase, followed by a decrease in dry weight (Vrabl et al. 2019).

In Figure 3, it can be seen that *C. lunata* grow better on the tofu liquid waste media, although the conidia density is lower when compared to other fungi. It is suspected that the nutrient content in tofu liquid waste plays an important role in supporting the growth of *C. lunata* when compared

to other liquid media. This condition is under the opinion of Meletiadis et al. (2001) and De Ligne et al. (2019). According to Prajapati et al. (2016), among the liquid media, oatmeal broth, potato dextrose broth, and Richards broth were the best liquid media for the growth and sporing of *C. eragrostidis*. Also, the differences in the growth of

the tested pathogenic fungi in the same liquid media were thought to be caused by various fungal genetic factors. In this context, Gałazka & Grządziel (2018) stated that the three aspects of microbial diversity are species, genetics, and function. The genetic structure of fungi is significantly associated with cultivation and the season.

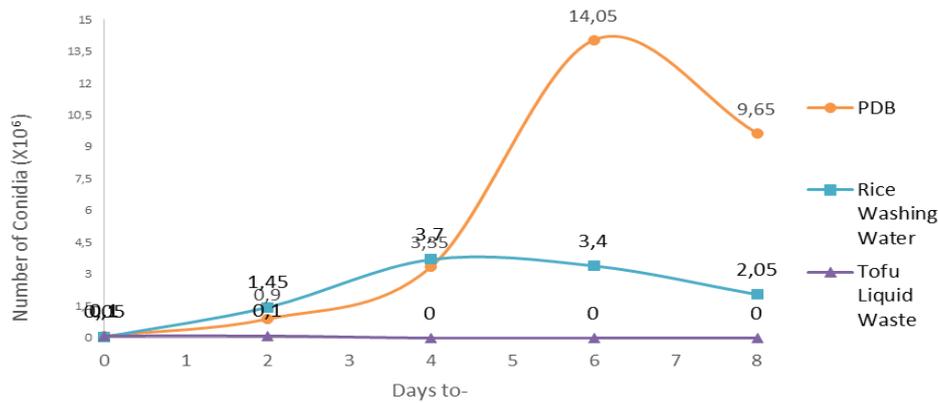


Figure 1. Growth of *Chaetomium* sp. on several liquid media

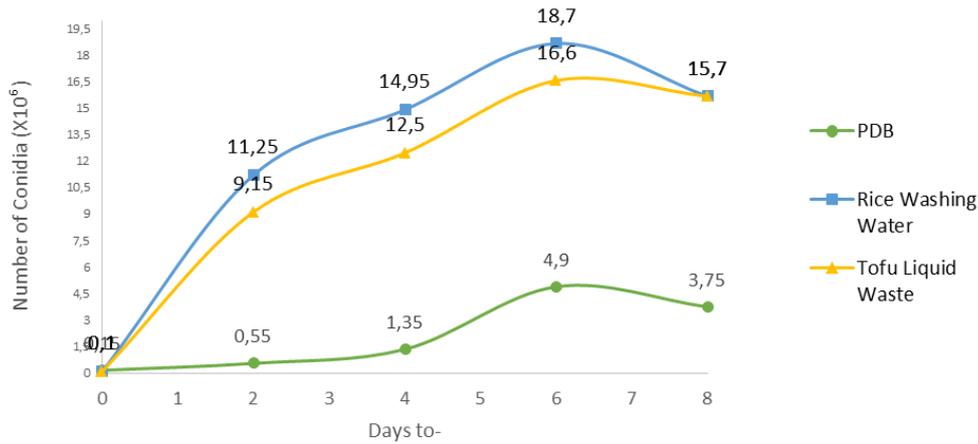


Figure 2. Growth of *Fusarium* sp. on several liquid media

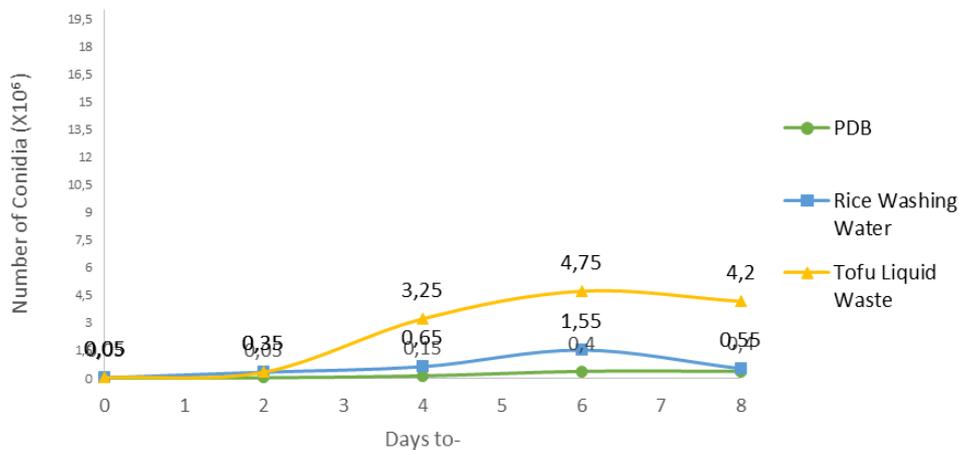


Figure 3. Growth of *Curvularia lunata* on several liquid media

The ability test of pathogenic fungi to multiply on alternative liquid media against weeds in planta

The results of the application of several weed pathogenic fungi to several types of in plant weeds are shown in Table 2. The data showed that the weed pathogenic fungi had different variations in promoting the disease and that had a significant effect on the pathosystem components and the number of leaves, but not significantly different on height.

Single treatment of fungi showed significant differences in the incubation period variables, disease intensity, AUDPC, and the number of leaves; while plant height not significantly different (Table 2). The acceleration of the incubation period was shown by the *Chaetomium* sp. amounted to 36.03% compared to control and not significantly different from *C. lunata* by 32.99%. This condition corresponds to the role of the two fungi that cause disease in the leaves. Following the opinion of Guo et al. (2016) and Tann and Soyong (2017), that *Chaetomium* sp. and *C. lunata* is a fungal pathogen that causes leaf disease. Srivastava et al. (2020) also reported that *C. lunata* is a major cause of leaf spot disease or blight in plants, which is economically important and causes major economic losses.

The acceleration of the appearance of symptoms is in line with the increase in disease intensity in weeds due to *Chaetomium* sp. and *C. lunata*, which were not significantly different, respectively 85.32 and 83.23%; and had an impact on the AUDPC increase of 88.29 and

84.67% for *Chaetomium* sp. and *C. lunata*, respectively, however *Chaetomium* sp. caused a reduction in the number of leaves by 33.07%, which was significantly different from the control and other pathogenic fungi. This shows that *Chaetomium* sp. more virulent than *C. lunata*, and other tested fungi. According to Harding and Raizada (2015) and Mohammed & Badawy (2020), this is that *Chaetomium* sp. widely used as a biological agent to control weeds. This condition due to the *Chaetomium* sp. produces secondary metabolite compounds, including chaetoglobosin, which has broad biological activities including antifungal, antibacterial, phytotoxins, and nematocides (Li et al. 2014; Chen et al. 2020).

Table 2 appears that the type of weed most susceptible to attack by weed pathogens is *A. conyzoides*. Pathogenic fungal infections in *A. conyzoides* weeds caused rapid symptoms of disease appearing or an incubation period, which is not significantly different from other weeds, but significantly different from *C. kyllingia* and *P. oleracea* weeds. This is consistent with the intensity of the disease and the AUDPC value. Research results from Sulandari et al. (2006), demonstrated that *A. conyzoides* is one of the weeds that are very susceptible to viral infections. *A. conyzoides* are also susceptible to infection with powdery mildew pathogens (Mukhtar & van Peer 2017; Hera et al. 2018). Meanwhile, for the variable plant height, *P. oleracea* grass shows a higher plant height than other weeds (Table 2). This is presumably not due to the low attack of pathogenic fungi, but rather due to genetic factors.

Table 2. Effects of single and combined types of fungi and weed types on the test in planta

Treatments	Incubation period (dai)	Disease intensity (%)	AUDPC (%-days)	Number of leaves	Plant height
Weed pathogenic fungi					
Control	32,83 b	2,25 a	3,20 a	51,22 b	53,08 a
<i>Chaetomium</i> sp.	21,00 a	15,33 b	27,33 c	34,28 a	37,50 a
<i>Fusarium</i> sp	28,17 ab	6,58 a	12,00 ab	49,50 ab	53,94 a
<i>Curvularia lunata</i>	22,00 a	13,42 b	20,87 bc	34,81 ab	44,14 a
Kinds of weed					
<i>C. kyllingia</i>	33,17 b	12,93 a	10,25 ab	33,03 ab	37,33 a
<i>C. dactylon</i>	32,58 b	6,87 a	3,45 a	32,11 a	78,42 b
<i>P. oleracea</i>	20,00 a	13,55 a	13,58 b	52,42 b	41,22 a
<i>A. conyzoides</i>	18,25 a	27,26 b	36,12 c	52,25 b	31,69 a
Combination of the pathogenic fungi and kinds of weed					
Contr- <i>C. kyllingia</i>	- e	0,00 a	0,00 a	25,89 ab	38,56 a
Contr- <i>C. dactylon</i>	- e	0,00 a	0,00 a	25,44 aa	84,89 a
Contr- <i>P. oleracea</i>	- e	0,00 a	0,00 a	72,22 g	44,00 a
Contr- <i>A. conyzoides</i>	26,33 cde	9,00 abc	12,83 a	81,33 g	44,89 a
<i>Chaeto-C. kyllingia</i>	33,00 e	16,67 bc	20,66 a	28,56 ab	32,56 a
<i>Chaeto-C. dactylon</i>	- e	0,00 a	0,00 a	27,67 ab	57,22 a
<i>Chaeto-P. oleracea</i>	6,00 a	10,00 abc	22,00 a	49,00 de	38,44 a
<i>Chaeto-A. conyzoides</i>	10,00 ab	34,67 c	66,67 b	31,89 abc	21,78 a
<i>Fusa-C. kyllingia</i>	- e	0,00 a	0,00 a	42,33 bcc	44,64 a
<i>Fusa-C. dactylon</i>	- e	0,00 a	5,83 a	46,44 cde	95,89 a
<i>Fusa-P. oleracea</i>	33,00 e	8,33 ab	11,17 a	62,44 fg	45,22 a
<i>Fusa-A. conyzoides</i>	17,33 bc	18,00 bc	31,00 ab	46,78 def	30,00 a
<i>Curvu-C. kyllingia</i>	29,67 e	13,33 abc	20,33 a	35,33 ef	33,56 a
<i>Curvu-C. dactylon</i>	33,00 e	6,67 ab	8,00 a	28,89 abc	75,67 a
<i>Curvu-P. oleracea</i>	6,00 a	6,33 ab	21,17 a	26,00 ab	37,22 a
<i>Curvu-A. conyzoides</i>	19,33 bcd	27,33 bc	34,00 a	49,00 def	30,11 a

Note: The number followed by the same letter in the same column shows no significantly different according to the 5% DMRT level. dai= days after inoculation.

The combined analysis of pathogenic fungi and weed species was significantly different (Table 2). The pathogenic fungus *Chaetomium* sp. Was able to infect and cause disease symptoms in *C. kyllingia* and *A. conyzoides*; while the pathogenic fungus *Fusarium* sp. and *C. lunata* was only able to infect *A. conyzoides*. This can be seen in the incubation period and disease intensity data, although the AUDPC data were not significantly different for the two fungi compared to *Chaetomium* sp. *Chaetomium* sp. is a fungus that is virulent to *A. conyzoides*. This is consistent with the research results of Mukhtar & van Peer (2017) and Hera et al. (2018).

The results showed that rice washing water and tofu liquid waste were suitable media for propagating weed pathogenic fungi. *Chaetomium* sp. is a fungal pathogen for broadleaf weeds, especially against *A. conyzoides* weeds. This fungus can be promoted as an ingredient for the manufacture of organic herbicides broadleaf weeds, especially *A. conyzoides* weeds.

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