J. Trop. Plant Pests Dis.
Vol. 23, No. 2, September 2023
Pages: 31–41

ISSN: 1411-7525
E-ISSN: 2461-0399
DOI : 10.23960/j.hptt.22331–41

RESEARCH PAPER

Preservation of weeds' pathogenic fungi in tempeh and tapioca liquid waste and its effectiveness in goatweed (*Ageratum conyzoides*)

Loekas Soesanto[1], Endang Mugiastuti[1], Murti Wisnu Ragil Sastyawan[2], & Abdul Manan[1]

Manuscript received: 26 December 2022. Revision accepted: 10 February 2023. Available online: 28 June 2023.

ABSTRACT

This research aimed to determine the best liquid media for the propagation of weed pathogenic fungi, the duration of the fungus storage on the media, and their virulence on goatweed (*Ageratum conyzoides*). The research consisted of two stages, i.e., the propagation of weed pathogenic fungi in alternative liquid media using a factorial completely randomized design, with the first factor being the pathogenic weed fungus (*Curvularia* sp., *Fusarium* sp., and *Chaetomium* sp.) and the second one being the media (tempeh or tapioca liquid waste) with four replicates. Applications were carried out using a hand sprayer on the underside of weed leaves at a density of 10^6 conidia or cfu mL^{-1}. Each weed was sprayed with 10 mL of the solution. Variables observed were conidia density, number of colonies, incubation period and symptoms, disease intensity, the area under the disease progress curve (AUDPC), plant height, number of leaves, dry crown weight, and dry root weight. The results showed that tempeh and tapioca liquid waste media could be used as alternative media for multiplying pathogenic fungi. The conidia density of *Curvularia* sp. was 2.375×10^9 conidia mL^{-1} higher than that of *Fusarium* sp. at 1.7×10^9 conidia mL^{-1} and *Chaetomium* sp. at 9.5×10^4 cfu mL^{-1}. *Curvularia* sp. propagated in tempeh liquid waste was able to cause damage to the leaves of goatweed as shown successively from the incubation period of 3.33 dai or accelerating 81.50%, increasing the disease intensity of 88.78%, and the AUDPC of 713.25% days compared to control. The most effective shelf life of *Curvularia* sp., *Fusarium* sp., and *Chaetomium* sp. in both tempeh and tapioca liquid waste media was found at six weeks at room temperature. *Curvularia* sp. in tapioca liquid waste could decrease weed height, the number of leaves, shoot dry weight, and root dry weight by 45.11, 28.65, 22.12, and 46.25%, respectively, compared to control.

Key words: goatweed, long shelf life, waste liquid media, weed pathogenic fungi

INTRODUCTION

Indonesia is a tropical country with fertile soil and high biodiversity. Indonesian soil, with its fertility, will produce plants that can grow wild or grow by being planted and given care. Weeds are yield-limiting factors besides pests and pathogens. Weeds thrive alongside the main crop, whose presence is not expected by farmers because it interferes with plants (Raza et al., 2019). Goatweed (*Ageratum conyzoides*) is a weed that generally becomes the dominant weed in various crop cultivation areas (Pyšek et al., 2004).

Weeds cause a decrease in both the quality and quantity of a plant and slow plant growth (Raza et al., 2019). Losses caused by weeds are usually caused by the nature of weeds that have high competitiveness (Abouziena et al., 2014-2015). The nature of weeds that have high competitiveness is typically what causes losses (Abouziena et al., 2014-2015). Farmers prefer chemical weed control that relies on herbicides so far because it produces results quickly (Shamkuwar et al., 2019; Singh et al., 2020). The use of herbicides has a negative effect on cultivated plants (Cullen et al., 2019), so efforts are made to look for compounds that are selective and applied in the right way.

The continuous use of chemical herbicides has a negative effect on the environment, causing weeds to become resistant and triggering the emergence of new, more aggressive weeds (Rahman, 2020; Ustuner et al., 2020). This control method in the future will face many challenges because the development of herbicides is faced with the need for more specific chemical compounds with increasing development costs and decreasing demand (Duke et al., 2022). Biological control, with the use of plant pathogens, provides an alternative to the use of chemical control, because it is effective, safe, selective, and practical (He et al., 2021).

Weed-pathogenic fungi are an alternative to natural weed control. Three weed pathogenic fungi have been explored and identified: *Curvularia* sp., *Fusarium* sp., and *Chaetomium* sp. (Soesanto et al.,

Corresponding author:
Loekas Soesanto (lukassusanto26@gmail.com)

[1]Faculty of Agriculture, Jenderal Soedirman University, Purwokerto, Central Java, Indonesia 53123
[2]Faculty of Technique, Diponegoro University, Semarang, Central Java, Indonesia 50275

2020). The three fungi have been tested against several types of weeds (Ziaulhak et al., 2019; Soesanto et al., 2020; 2021). Propagation of pathogenic fungi can use alternative, easy, and inexpensive media (Basu et al., 2015; Soesanto et al., 2021). Organic liquid waste has excellent potential as a medium for the propagation of biological agents because it contains a good nutritional composition for microbial growth (Siddeeg et al., 2020). Among them are tempeh and tapioca liquid wastes. The protein, carbohydrates, and fat content in the liquid waste of tempeh were 0.47 g, 4.06 g, and 0.04 g, respectively (Sari & Rahmawati, 2020). Tapioca wastewater has a carbon content of 119.11 mgL^{-1} (Kunindar et al., 2018). The purpose of this study was to determine the best organic liquid media for the propagation of weed pathogenic fungi, their virulence against weeds, and the effective shelf life of these fungi cultivated in tempeh liquid waste or liquid waste of tapioca.

MATERIALS AND METHODS

Research Site. The research was conducted at the experimental farm, Faculty of Agriculture, Jenderal Soedirman University, in Purwokerto Central Java, at 125 m above sea level.

Preparation of the Pathogenic Fungi Isolates. The fungi isolates identified as *Fusarium* sp., *Chaetomium* sp., and *Curvularia* sp.. They were the result of exploration from previous studies and had undergone a series of tests (Soesanto et al., 2020). Each pathogenic weed fungus was then propagated on PDA media in Petri dishes and incubated for seven days at room temperature (Alsohaili & Bani-Hasan, 2018).

Preparation of Liquid Media. This tempeh liquid waste was obtained from the tempeh industrial center, Pliken Village, Kembaran District, Banyumas Regency, Central Java Province, Indonesia. The tapioca liquid waste was obtained from the factory at Manonjaya District, Tasikmalaya Regency, West Java Province, Indonesia. Tempeh liquid waste and tapioca liquid waste were filtered first using filter paper. The pH of each liquid medium was measured and adjusted to be neutral (pH 7) using HCl or NaOH. Furthermore, tempeh and tapioca liquid waste were boiled with 10 gL^{-1} of sugar. After that, the media was sterilized using an autoclave for 30 min at 121 ºC (Soesanto et al., 2021).

Propagation of the Pathogenic Fungi Isolates. Pathogenic fungi were propagated using tempeh liquid waste and tapioca liquid waste, which had been sterilized. The liquid media used was 100 mL in each 250 mL Erlenmeyer flask. Inoculation was done by adding 5 cork drill bits (diameter 1.0 cm) colonies of each fungal isolate. The inoculated media was then incubated for 7 days using a shaker at 150 rpm at room temperature (Hudson et al., 2021). Next, the Erlenmeyer flask was removed from the shaker and stored for 12 weeks at room temperature to determine the shelf life of each fungal isolate. Every 10 days, the conidia density was calculated for *Fusarium* sp. and *Curvularia* sp., which were present in each medium from inoculation until 10 weeks after inoculation using a hemocytometer to obtain a density of 10^6 conidia mL^{-1} (Kamaruzzaman et al., 2016). Meanwhile, specifically for *Chaetomium* sp., which has been propagated in tempeh liquid waste media and liquid tapioca waste, dilutions were carried out in stages up to 10^{-3} cfu $mL^{-1.}$ The fungus is propagated differently because it is difficult to produce conidia when grown in liquid waste. Fungal colonies growing from isolation were counted using the Total Plate Count (TPC) method to obtain a density of 10^6 cfu mL^{-1} (Parveen et al., 2014).

Preparation of Goatweed. Preparation was carried out by exploring uniform weed seedlings with a height of 11 cm and a number of leaves 7 strands. Weeds were planted in 15 × 25 cm polybag media in the screen house; each polybag contained three weeds.

Application of the Pathogenic Fungi. The method of application of weed pathogenic fungi was a liquid waste that had been given and preserved for 8 weeks. Applications were carried out using a hand sprayer on the underside of weed leaves at a density of 10^6 conidia or cfu mL^{-1}. Each weed was sprayed with 10 mL of solution without adding stickers, and then each weed was covered with plastic for 1 × 24 hours before the plastic was discarded. The application was carried out five times with an interval of three days.

Experimental Design. The study consisted of two stages, the first stage The study consisted of two stages, the first stage was propagation of weed pathogenic fungi in alternative liquid media. The test used a factorial completely randomized design. The first factor was the type of weed pathogenic fungi (*Curvularia* sp., *Fusarium* sp., and *Chaetomium* sp.) and the second factor was the type of liquid media (tempeh liquid waste and tapioca liquid waste). All treatments were replicated four times. The second stage of research was the spraying application on goatweed with a randomized block design consisting of control (goatweed not

inoculated), *Chaetomium* sp. + tempeh liquid waste, *Fusarium* sp. + tempeh liquid waste, *Curvularia* sp. + tempeh liquid waste, *Chaetomium* sp. + tapioca liquid waste, *Fusarium* sp. + tapioca liquid waste, or *Curvularia* sp. + tapioca waste, with four replications.

Variables and Measurements. Variable observations carried out in *in vitro* tests were measurements of fungal conidia density. Counting was carried out every 10 days to obtain a relatively constant (stationary) density using a hemocytometer (Kamaruzzaman et al., 2016).

Whereas, in screen house trials, observation was made on disease intensity, which was carried out twice a week for three weeks. The intensity of the disease was calculated following the formula (Wongpia & Lomthaisong, 2010):

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

DI = Disease intensity (%);
v = Value of the measurement results of the unit of observation;
Z = Highest numerical value in the category of damage;
N = Number of plants.

Symptom level of infection description (Bhat et al., 2013):
0 = No leaf infection;
1 = Infected leaf area 0–10%;
2 = Infected leaf area >11–25%;
3 = Infected leaf area >26–45%;
4 = Infected leaf area >46–75%;
5 = Leaf area infected >76%.

The value of area under the disease progress curve (AUDPC) was obtained from observing disease incidence. AUDPC can be calculated following the formula (Simko & Piepho, 2012):

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

AUDPC= Disease progression curve (%-days);
Y = i+1 observation data;
Y_i = 1st observation data;
T_{i+1} = i+1 observation time;
T_i = 1st observation time.

Plant height measurements were carried out twice, at the beginning and end of the study. The number of leaves per plant was calculated from the initial conditions before treatment until the last week of observation. The fresh crown weight was measured after the completion of digestion. The weed fresh weight measurement was carried out using an analytical balance. The dry crown weight was measured after roasting. Weed weight measurement was carried out using an analytical balance. Fresh root weight was measured after the completion of destruction. Dry root weight was measured after oven drying.

RESULTS AND DISCUSSION

Propagation of the Pathogenic Fungi in the Liquid Waste. The single media treatment had no significant effect on the conidia density variable of the fungus (Table 1). This means that tempeh liquid waste and tapioca liquid waste have the same effect, and both can be used as alternative media to replace PDB. The organic waste content can support the growth and development of pathogenic fungi at the time of research. Tempeh liquid waste is thought to have good nutrient content to be used as a medium for fungal growth (Hartini et al., 2018). Tempeh liquid waste contains (C) = 8,51% and Nitrogen (N) = 2,27%, with a C/N value = 3.76%, C/N value 1.5–2.5% is a critical threshold, while the optimum C/N value is 25–30% (Puyuelo et al., 2011). Thus, the organic matter contained in tempeh liquid waste has been decomposed to be used as a growth supplement.

The single pathogen treatment significantly affected the variable density of fungal conidia (Table 1). The conidia density of *Curvularia* sp. was 28.42% higher than *Fusarium* sp. and 99.9% higher than *Chaetomium* sp.. *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 the propagation of biological agents (Alibardi et al., 2020) because it contains a good nutritional composition for microbial growth, such as carbohydrates, proteins, water, amino acids, fats, mineral salts, and other nutrients (Adebayo & Obiekezie, 2018).

Media requirements for the growth of *Curvularia* sp. are different from the growing conditions of *Fusarium* sp., *Curvularia* sp., and *Fusarium* sp. are included in the soil-borne fungus, but the infection of the two fungi differs. *Curvularia* sp. more infects on the surface, especially in the leaves, while *Fusarium* sp. generally infects plant tissues, especially plant roots (Basu et al., 2015). Meanwhile, *Chaetomium* sp. had the lowest conidia density, presumably because the composition of the compounds in the organic liquid medium did not match the needs of the fungus to grow (Soesanto et al., 2021).

Based on the analysis of media × pathogenic fungi variety (Table 1), the conidia density in the

tempeh liquid waste + *Curvularia* sp. is 42.85% higher than tapioca liquid waste + *Curvularia* sp.. Therefore, *Curvularia* sp. grows better in tempeh liquid waste than in tapioca liquid waste. This can happen because tempeh liquid waste contains better nutrients than tapioca liquid waste. If this fungus lives on dissolved organic compounds, it is a saprophytic fungus. In addition, tempeh liquid waste contains nitrogen elements needed by pathogenic fungi to grow and develop (Chaerun, 2009). In the opinion of Manan et al. (2021), the nitrogen content of the substrate influences the growth and development of the fungal isolate mycelia.

Meanwhile, *Chaetomium* sp. grown on organic liquid waste did not grow well. The growth rate is the lowest compared to the development of the other two pathogenic fungi. This is primarily determined by the genetic nature of the fungus, which affects the habits of the fungus to live, especially the nutrients needed to live (Bonfante & Desirò, 2017). This condition also encourages the adaptation of fungi. Fungi that can adapt to new environments, especially new nutrients to support their growth, will survive and utilize these nutrients for growth (Jacoby et al., 2017). The ability of fungi to grow considerably determines the infection and pathogenicity of fungi in plants. In addition, the ability of fungi to grow well also determines the opportunity for fungi to find the infection site in plants (Doehlemann et al., 2017).

Preservation Time. The development of conidia density in the two liquid media produced the development curve of weed pathogenic fungi (Figures 1, 2, and 3). In the *Curvularia* sp. curve, it can be seen that the highest fungal growth was at week 6 in both tempeh and tapioca liquid waste (Figure 1). In this phase, the fungus has adapted to the new organic nutrients so that it can grow well and enter the exponential stage. The speed of fungal growth is strongly influenced by physical factors and the growing medium, such as pH and nutrient content, as well as environmental conditions, including temperature and humidity (Ali et al., 2017). In addition, the growth speed is also determined by the nutrient content of the growth medium (Basu et al., 2015). Proper nutrition will support fungal growth. Based on the graphs (Figures 1 and 2), the good shelf life is in the 6th week. After more than six weeks, the density decreases, which reduces the level of pathogenicity. The decline in fungal growth after entering the exponential stage was caused by several things, including the depletion of nutrients in the growing medium due to competition for nutrients. The fewer nutrients, the slower the growth of fungi because of the higher competition for nutrients (Vrabl et al., 2019).

A description of the growth curve on *Fusarium* sp. showed the highest growth in both tempeh and tapioca liquid waste, namely in the 6th week (Figure 2). This is consistent with the growth of *Curvularia* sp. beginning of growth until the 4th week, the fungus undergoes an adaptation or log phase, and which

Table 1. Results of weed pathogenic fungus density after a shelf life of 10 weeks

Treatments	Conidia density (x 10^2 conidia mL^{-1})	
Media		
Tempeh liquid waste	7,983,373	a
Tapioca liquid waste	1,183,335	ab
Pathogenic fungi		
Curvularia sp.	23,750,000	c
Fusarium sp.	17,000,000	b
Chaetomium sp.	950	a
Media × Pathogenic fungi		
Chaetomium sp. + Tempeh liquid waste	3	ab
Fusarium sp. + Tempeh liquid waste	248,750	c
Curvularia sp. + Tempeh liquid waste	350,000	cd
Chaetomium sp. + Tapioca liquid waste	1.25	a
Fusarium sp. + Tapioca liquid waste	687,500	e
Curvularia sp. + Tapioca liquid waste	200,000	bc

Numbers followed by different letters in the same row indicate that they are significantly different at 5% level of DMRT.

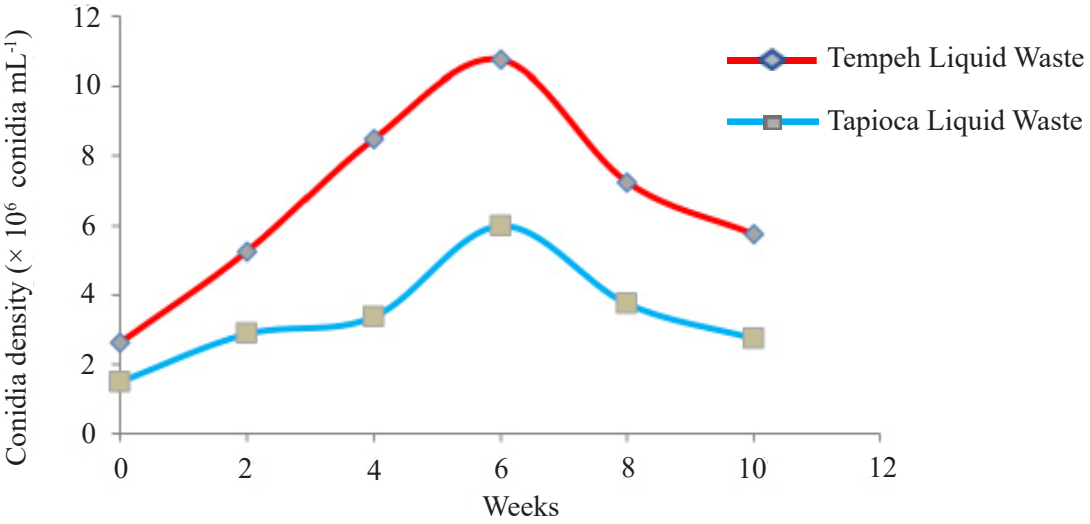

Figure 1. Conidia density of the fungus *Curvularia* sp. on liquid waste media.

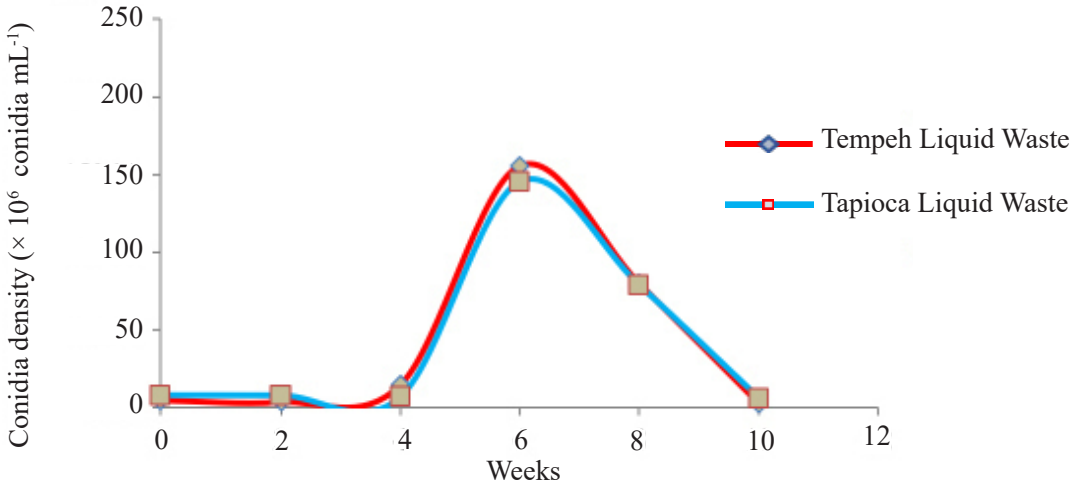

Figure 2. Density of conidia of *Fusarium* sp. in liquid waste media.

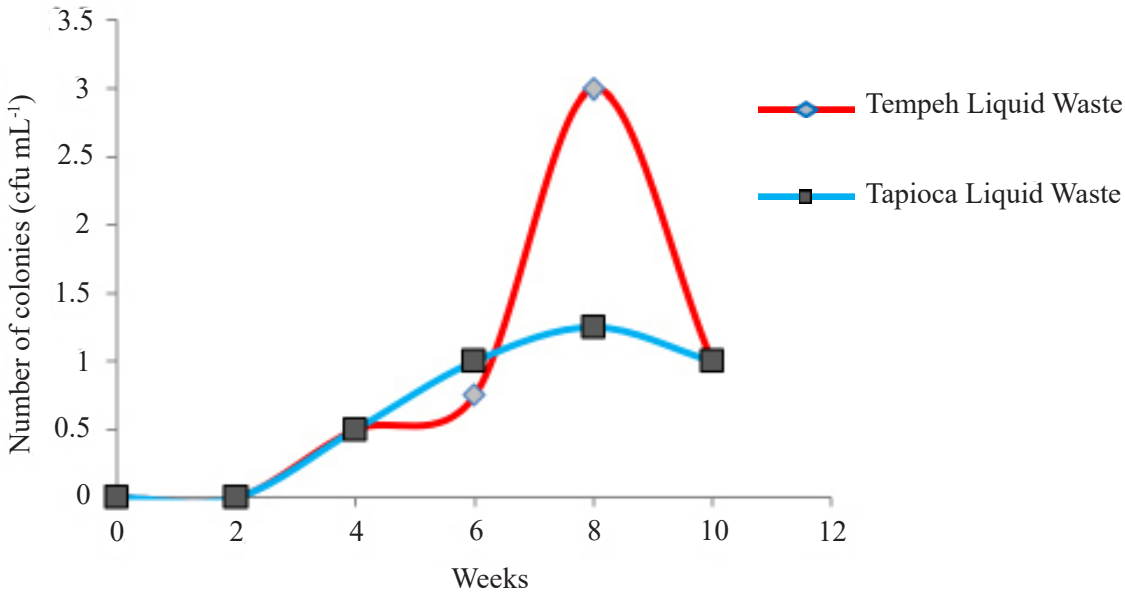

Figure 3. Number of colonies of *Chaetomium* sp. in liquid waste media.

different from the initial growth of *Culvularia* sp. (Figure 1), which did not have an adaptation stage when grown on new organic medium. *Fusarium* sp. takes 4 weeks to adapt to new organic medium. It is suspected that the new organic media is more suitable for the growth of *Curvularia* sp. than *Fusarium* sp.

Fusarium sp. is a soil-borne fungus that requires nutrients for its growth in the form of proteins and other readily utilized compounds (Basu et al., 2015). *Fusarium* sp. is not equipped with enzymes to degrade organic matter, so the ability to use liquid organic matter is minimal. The information in Table 1 illustrates and supports this. In contrast to *Curvularia* sp., the nutritional needs of *Curvularia* sp. are needed to grow. This indicates that *Curvularia* sp. is a fungus that is easy to grow on new organic nutrients compared to the other two fungal species tested (Pan et al., 2018). In Figure 2, the two liquid wastes are suitable for the growth of *Fusarium* sp. The nutrient content of each liquid waste determines this. Tempeh liquid waste and tapioca liquid waste contain nutrients needed for the growth of *Fusarium* sp. and will affect the high density of fungi, causing more and more fungi to form new conidia (Chaerun, 2009; Kunindar et al., 2018).

Based on the growth curve, the conidia density of *Fusarium* sp. decreased from the 6th to 10th week. This decrease is caused by the competition between conidia for nutrients. In addition, the formation of secondary compounds from conidia, which are fungal secondary metabolites toxic to fungal conidia, causes the conidia to die. Decreasing the density of conidia will also result in a decrease in the level of pathogenicity of *Fusarium* sp. According to Uysal & Kurt (2017), inoculum concentration influences infection and lesion development. Although the number of conidia of *Fusarium* sp. was low, the pathogenicity will still be high if the fungus has a high virulence gene. In the stationary phase, the number of cells will decrease with reduced nutrients in the medium or the presence of toxic compounds and decrease with increasing time (Pletnev et al., 2015).

Based on the growth curve, *Chaetomium* sp. showed that the growth of *Chaetomium* sp. was highest in week 8 for both tempeh and tapioca wastewater (Figure 3). *Chaetomium* sp. requires a shorter adaptation time than *Fusarium* sp., which is only two weeks, and then grows into an exponential stadium. The peak growth of *Cahetomium* sp. in the 8th week will decrease in growth. *Chaetomium* sp.'s highest growth also occurs in tempeh liquid waste, which shows that in tempeh liquid waste, the nutritional content is more complete and more in line with *Chaetomium* sp. compared to nutrients in tapioca liquid waste.

According to Uikey et al. (2020), the choice of growth medium has a significant impact on the colony diameter, characters (texture, surface, reverse coloration, and zoning), and sporulation of test fungi. The decrease in conidia was due to the long shelf life of solid media and the content contained in tempeh and tapioca liquid waste. This is in line with the research of Long et al. (2017) as the shelf life increases, the percentage of conidial germination production tends to decrease.

Pathosystem Components of Goatweed. The most extended incubation period was tempeh + *Curvularia* sp. and tapioca liquid waste + *Fusarium* sp. (Table 2). The incubation period in the treatment of *Curvularia* sp. and *Fusarium* sp. showed a significant difference compared to the control. The incubation period in *Curvularia* sp. and tempeh liquid waste was 81.50% faster than the control (Table 2). The short incubation period is thought to be due to several factors, such as the aggressiveness of the pathogen in causing disease and the absence of inhibition of the pathogen by other microbes (van

Table 2. Effect of alternative media + pathogenic fungi on pathosystem components

Treatments	Incubation period (dai)	Disease intensity (%)	AUDPC (87%-days)
Control (goatweed not inoculated)	18.00 a	0 a	0 a
Chaetomium sp. + Tempeh liquid waste	5.50 bc	80.40 e	358.08 b
Fusarium sp. + Tempeh liquid waste	6.67 c	70.58 b	296.83 b
Curvularia sp. + Tempeh liquid waste	3.33 a	88.78 g	713.25 c
Chaetomium sp. + Tapioca liquid waste	5.33 bc	71.11 c	349.86 b
Fusarium sp. + Tapioca liquid waste	3.33 a	82.76 f	386.58 b
Curvularia sp. + Tapioca liquid waste	4.25 ab	79.45 d	361.86 b

Note: Numbers followed by different letters indicated significantly different with DMRT at an error level of 5%; dai = days after inoculation.

Seventer & Hochberg, 2017). In addition, also a large number of microbial populations will infect more quickly.

The incubation period for the control goatweed was 18 days after inoculation because the control weeds until the end of the observation showed no disease symptoms due to the three weed pathogenic fungi. Symptoms of the disease appear more quickly in the inoculation of *Curvularia* sp. and *Fusarium* sp., either grown in tempeh or tapioca liquid waste, respectively, which is thought to be caused by the high population of fungal conidia produced. This agrees with the conidia density (Table 1). The interaction of weed-pathogenic fungi and organic liquid waste will determine the ability of weed-pathogenic fungi to infect goatweed or their virulence. This is evidenced by the interaction of each weed pathogenic fungus with each organic liquid waste (Table 2). Even though all the interaction results can speed up the incubation period compared to the control, the time needed will differ. This situation indicates that the nutrients in the growth media will determine the ability of microbial infection. The presence of these nutrients will encourage the production of microbial secondary metabolites, which play an essential role in the microbial infection of host plants.

The disease intensity in the treatment showed a significant difference when compared to the intensity of the disease in the controls (Table 2). The disease intensity in tempeh liquid waste + *Curvularia* sp. was 88.78% higher than the control and 10.51% higher than tapioca liquid waste. This is consistent with the incubation period data. That is, *Curvularia* sp. in tempeh liquid waste has a better ability to infect host plants, which is shown to be the highest disease intensity compared to control and tapioca liquid waste + *Curvularia* sp. as well as compared with the two other weed pathogenic fungi. Meanwhile, *Fusarium* sp. in tapioca liquid waste showed a higher intensity of 82.76% compared to the control and 14.72% compared to tempeh liquid waste. *Fusarium* sp. prefers tapioca liquid waste to tempeh liquid waste, consistent with Figure 2. This is due to the nutrients in tapioca liquid waste needed by *Fusarium* sp. to grow and produce more microconidia populations, making it easier to infect host plants and have higher disease intensity values.

Disease intensity due to inoculation of *Chaetomium* sp. in tempeh liquid waste showed higher data when compared to the control and tapioca liquid waste. This condition is similar to *Curvularia* sp., which is more suitable for growing on tempeh liquid waste than tapioca liquid waste. This is because the nutrients in the tempeh liquid waste are preferred by the two fungi for the growth and production of conidia.

AUDPC in the treatment showed significantly different results when compared to AUDPC in the control (Table 2). This is consistent with data on disease intensity and incubation period which are also significantly different. AUDPC is used to determine the relationship between disease intensity and time. Based on Table 2, AUDPC in tempeh liquid waste + *Curvularia* sp. showed a higher value of 713.25% compared to the control and 49.27% higher than the tapioca liquid waste + *Curvularia* sp. In line with the disease intensity data, the AUDPC value on *Fusarium* sp. in tapioca liquid waste is higher than in tempeh liquid waste.

In contrast, in *Chaetomium* sp., the AUDPC value is also consistent with disease intensity. AUDPC value indicates the value of disease progression in units of time. The AUDPC value is also affected by environmental factors, especially temperature, and humidity. Temperature and humidity are suitable for the growth of goatweed, supported by the high virulence of the weed's pathogenic fungi, will promote high disease development (Steketee et al., 2016). The higher the AUDPC value, the higher the disease intensity and the higher the disease progression. The higher the AUDPC value indicates, the higher the plant resistance and more stable genotype across environments. Conversely, the lower the AUDPC value, the lower the disease development (Bocianowski et al., 2020).

Growth Component of Goatweed. Weed height and number of leaves were significantly affected by treatment (Table 3). This shows that the differences in weed pathogenic fungi and the two liquid wastes can affect weed height and the number of leaves. The initial weed material used has been sought to be uniform so that the existing differences are only due to the effect of the treatment. *Curvularia* sp. in tapioca liquid waste reduced weed height by 45.11% and the number of leaves by 28.65% compared to the control. This is presumably because *Culvularia* sp. was able to produce bioactive compounds in tapioca liquid waste media, affecting weed physiology. The physiological processes of weeds are disrupted and will result in stunted growth. The development of plant diseases can disrupt plant physiology, which causes the number of leaves to decrease and plant height does not to increase.

However, the application of *Fusarium* sp. in tempe liquid waste showed no significant difference in weed height and number of leaves compared to the control. It is suspected that *Fusarium* sp. in tempe or tapioca liquid waste, which causes wilting symptoms in weeds and does not drop leaves so that weed height and number of leaves are not reduced. This is in accordance

Table 3. Effect of pathogenic fungi and liquid waste on components of weed growth

Treatments	Incubation period (dai)	Disease intensity (%)	AUDPC (%-days)	Disease intensity (%)
Control (goatweed not inoculated)	10.33 de	10.75 bc	3.57 b	0.80 b
Chaetomium sp. + Tempeh liquid waste	7.17 b	9.33 b	1.35 a	0.25 a
Fusarium sp. + Tempeh liquid waste	9.67 de	9.58 b	2.58 a	0.59 a
Curvularia sp. + Tempeh liquid waste	9.08 d	19.00 c	3.30 ab	0.47 a
Chaetomium sp. + Tapioca liquid waste	7.42 bc	6.08 a	1.54 a	0.35 a
Fusarium sp. + Tapioca liquid waste	11.08 e	9.42 b	3.29 ab	1.05 b
Curvularia sp. + Tapioca liquid waste	5.67 a	7.67 a	1.61 a	0.43 a

Numbers followed by different letters in the same coloumn indicated significantly different according to DMRT with an error level of 5%.

with the opinion that *Fusarium* sp. causing the plants to wither and not drop the leaves. In contrast, the application of *Curvularia* sp. in tapioca liquid waste showed significant differences compared to the control and other treatments in weed height and number of leaves, i.e. 45.11 and 28.65%, respectively (Table 3).

Tempeh and tapioca liquid waste respectively to reproduce *Chaetomium* sp. gave less leaves than the control and other fungi. This is in line with the results of Sadh et al. (2018) that the tempeh industrial liquid waste contains nutrients that can be absorbed by plant roots. Waste from making tempeh is included in biodegradable waste, which is waste material that can be destroyed by microbes. Tempe waste contained Mg, Si, P, S, K, Ca, Mn, Fe, and Zn. K had the highest elemental content, followed by Ca, P, and Mg. The Tempe waste is composed of C, N, and S with a C/N ratio of 11.20 (Chaerun, 2009). Liquid tapioca waste contains phosphate nutrient that can influence cell division and fat formation (Amalah & Widyartini, 2018). Cell division is a process by which the cell duplicates itself either for growth and repair or for reproduction of organism (Robinson, 2021).

The application of weed pathogenic fungi and both organic liquid wastes differ significantly compared to control but has no effect on the dry weight of both shoots and roots of goatweed. The results on shoot dry weight were higher in tapioca liquid waste + *Curvularia* sp. as 22.12% compared to the control. Plant dry weight is largely determined by plant biomass. If plant biomass is attacked by pathogenic fungi, the biomass will decrease and affect plant dry weight (Demura & Ye, 2010).

The highest reduction in dry weight of weed roots was shown by the all application of tempeh and tapioca liquid waste with both *Chaetomium* sp. and *Curvularia* sp. (Table 3). This condition is in line with other growth components especially in tapioca liquid waste. Reduction in root dry weight due to application of *Curvularia* sp. in tapioca liquid waste by 46.25% compared to control. As Robinson (2021) said, the nutrient content in tapioca liquid waste is useful for supporting the growth of organisms, in this case *Curvularia* sp. *Curvularia* sp. can grow well in tapioca liquid waste. Application of tapioca liquid waste with *Curcularia* sp. inhibited weed growth and resulted in a decrease in dry weight of plants and roots.

CONCLUSIONS

Tempeh and tapioca liquid waste media can be used as alternative media for the multiplication of pathogenic fungi. The conidia density of *Curvularia* sp. is 2.37×10^9 conidia mL^{-1} higher than that of *Fusarium* sp. at 1.7×10^9 conidia mL^{-1} and *Chaetomium* sp. at 9.5×10^4 cfu mL^{-1}. *Curvularia* sp. in tempeh liquid waste accelerated the incubation period and increased disease intensity and AUDPC by 81.50, 88.78, and 713.25%, respectively, compared to control. *Curvularia* sp. and tempeh liquid waste are able to cause damage to the leaves of goatweed, as shown successively from the incubation period of 3.33 dai, the disease intensity of 88.78 %, and the AUDPC of 713.25% days. The most effective shelf life of *Curvularia* sp., *Fusarium* sp., and *Chaetomium* sp. in both tempeh and tapioca liquid waste media is found at 6 weeks at room temperature. *Curvularia* sp. in tapioca liquid waste could decrease weed height, number of leaves, shoot dry weight, and root dry weight by 45.11, 28.65, 22.12, and 46.25%, respectively, compared to control.

ACKNOWLEDGMENTS

The authors thank to Directorate of Research

and Community Service, Deputy for Research and Development Enhancement, Ministry of Research and Technology/National Research and Innovation Agency for financial support through Applied Research Fund 2019-2021 year 2021. Thank goes to Alifia Muthia Salsabilla for her assistance.

FUNDING

The funding source of the research comes from Applied Research Fund 2021 and grants number of 203/SP2H/LT/DRPM/2021, date of March 18, 2021.

AUTHORS' CONTRIBUTIONS

LS and EM considered and planned the research; LS preparing the manuscript; LS and MWRS searching the publications and preparing the literatures; AM collecting and analyzing data. The authors provided response and comments on the research flow, data analysis and interpretation as well as shape of the manuscript. All the authors have read and approved the final manuscript.

COMPETING INTEREST

There is no competing interest regarding the publication.

REFERENCES

Abouziena HF, El-Saeid HM, & Amin ABAES. 2014-2015. Water loss by weeds: A review. *Int. J. ChemTech Res.* 7(01): 323–336.

Adebayo FO & Obiekezie SO. 2018. Microorganisms in waste management. *RJST.* 10(1) : 28–39. https://doi.org/10.5958/2349-2988.2018.00005.0

Ali SRM, Fradi AJ, & Al-Aaraji AM. 2017. Effect of some physical factors on growth of five fungal species. *Eur. Acad. Res.* V(2): 1069–1078.

Alibardi L, Astrup TF, Asunis F, Clarke WP, Gioannis GD, Dessì P, Lens PNL, Lavagnolo MC, Lombardi L, Muntoni A, Pivato A, Polettini A, Pomi R, Rossi A, Spagni A, & Spiga D. 2020. Organic waste biorefineries: Looking towards implementation. *Waste Manag.* 114: 274–286. https://doi.org/10.1016/j.wasman.2020.07.010

Alsohaili SA & Bani-Hasan BM. 2018. Morphological and molecular identification of fungi isolated from different environmental sources in Northern Eastern Desert of Jordan. *Jordan. J. Biol. Sci.* 11(3): 329–337.

Amalah N, Widyartini DS, Chistiani C, & Hidayah HP. 2018. The effect of dilution level of liquid tapioca waste culture medium and concentration of phosphate on the growth of microalgae *Navicula* sp.. *Nusantara Biosci.* 10(1): 64–68. https://doi.org/10.13057/nusbiosci/n100110

Basu S, Bose C, Ojha N, Das N, Das J, Pal M, & Khurana S. 2015. Evolution of bacterial and fungal growth media. *Bioinformation*. 11(4): 182–184. https://doi.org/10.6026/97320630011182

Bhat HA, Ahmed K, Ahangar RA, Qazi NA, Dar NA, & Ganie SA. 2013. Status and symptomatology of Alternaria leaf blight (*Alternaria alternata*) of Gerbera (*Gerbera jamisonni*) in Kashmir valley. *Afr. J. Agric. Res.* 8(9): 819–823.

Bocianowski J, Tratwal A, & Nowosad K. 2020. Genotype by environment interaction for area under the disease-progress curve (AUDPC) value in spring barley using additive main effects and multiplicative interaction model. *Australas. Plant Pathol.* 49: 525–529. https://doi.org/10.1007/s13313-020-00723-7

Bonfante P & Desirò A. 2017. Who lives in a fungus? The diversity, origins and functions of fungal endobacteria living in Mucoromycota. *ISME J.* 11: 1727–1735. https://doi.org/10.1038/ismej.2017.21

Chaerun SK. 2009. Tempeh waste as a natural, economical carbon and nutrient source: ED-XRF and NCS study. *HAYATI J. Biosciences.* 16(3): 120–122. https://doi.org/10.4308/hjb.16.3.120

Cullen MG, Thompson LJ, Carolan JC, Stout JC, & Stanley DA. 2019. Fungicides, herbicides and bees: A systematic review of existing research and methods. *PLoS ONE.* 14(12): e0225743. https://doi.org/10.1371/journal.pone.0225743

Demura T & Ye Z-H. 2010. Regulation of plant biomass production. *Curr. Opin. Plant Biol.* 13(3): 298–303. https://doi.org/10.1016/j.pbi.2010.03.002

Doehlemann G, Ökmen B, Zhu W, & Sharon A. 2017. Plant pathogenic fungi. Eukaryotes: Fungi and Parasitology. 5(1): 5.1.14. https://doi.org/10.1128/microbiolspec.FUNK-0023-2016

Duke SO, Pan Z, Bajsa-Hirschel J, & Boyette CD. 2022. The potential future roles of natural compounds and microbial bioherbicides in

weed management in crops. *Adv. Weed. Sci.* 40(spe1): e020210054. https://doi.org/10.51694/AdvWeedSci/2022;40:seventy-five003

Hartini S, Letsoin F, & Kristijanto AI. 2018. Productive liquid fertilizer from liquid waste tempeh industry as revealed by various EM4 concentration. *IOP Conf. Ser.: Mater. Sci. Eng.* 349: 012059. https://doi.org/10.1088/1757-899X/349/1/012059

He D-C, He M-H, Amalin DM, Liu W, Alvindia DG, & Zhan J. 2021. Biological control of plant diseases: An evolutionary and eco-economic consideration. *Pathogens*. 10(10): 1311. https://doi.org/10.3390/pathogens10101311

Hudson O, Waliullah S, Ji P, & Ali Md E. 2021. Molecular characterization of laboratory mutants of *Fusarium oxysporum* f. sp. *niveum* resistant to prothioconazole, a demethylation inhibitor (DMI) fungicide. *J. Fungi*. 7(9): 704. https://doi.org/10.3390/jof7090704

Jacoby R, Peukert M, Succurro A, Koprivova A, & Kopriva S. 2017. The role of soil microorganisms in plant mineral nutrition—Current knowledge and future directions. *Front. Plant Sci.* 8: 1617. https://doi.org/10.3389/fpls.2017.01617

Kamaruzzaman M, Hossain MD, & Hossain I. 2016. Antifungal and morphological assay of selective *Trichoderma* isolates against soilborne plant pathogenic fungi. *IJIAS.* 16(2): 409–417.

Kunindar S, Efendi E, & Supono. 2018. Utilization of tofu and tapioca industrial liquid waste for nile tilapia (*Oreochromis niloticus*) culture within different biofloc systems. *e-Jurnal Rekayasa dan Teknologi Budidaya Perairan.* 7(1): 763–773. https://doi.org/10.23960/jrtbp.v7i1.p763-774

Long NNV, Vasseur V, Couvert O, Coroller L, Burlot M, Rigalma K, & Mounier J. 2017. Modeling the effect of modified atmospheres on conidial germination of fungi from dairy foods. *Front. Microbiol*. 8: 2109. https://doi.org/10.3389/fmicb.2017.02109

Manan S, Ullah MW, Ul-Islam M, Atta OM, & Yang G. 2021. Synthesis and applications of fungal mycelium-based advanced functional materials. *J. Bioresour. Bioprod.* 6(1): 1–10. https://doi.org/10.1016/j.jobab.2021.01.001

Pan X, Qin Y, & Yuan Z. 2018. Potential of a halophyte-associated endophytic fungus for sustaining Chinese white poplar growth under salinity. *Symbiosis.* 76(2): 1–8. https://doi.org/10.1007/s13199-018-0541-8

Parveen S, Das S, Begum A, Sultana N, Hoque MM, & Ahmad I. 2014. Microbiological quality assessment of three selected spices in Bangladesh. *Int. Food Res. J.* 21(4): 1327–1330.

Pletnev P, Osterman I, Sergiev P, Bogdanov A, & Dontsova O. 2015. Survival guide: *Escherichia coli* in the stationary phase. *Acta Naturae*. 7(4): 22–33. PMC4717247.

Puyuelo B, Ponsá S, Gea T, & Sánchez A. 2011. Determining C/N ratios for typical organic wastes using biodegradable fractions. *Chemosphere.* 85(4): 653–659. https://doi.org/10.1016/j.chemosphere.2011.07.014

Pyšek P, Richardson DM, & Williamson M. 2004. Predicting and explaining plant invasions through analysis of source area floras: Some critical considerations. *Divers. Distrib*. 10(3): 179–187. https://doi.org/10.1111/j.1366-9516.2004.00079.x

Rahman MM. 2020. Potential environmental impacts of herbicides used in agriculture. *J. Agric. Forest Meteorol. Res*. 3(1): 266–269.

Raza A, Razzaq A, Mehmood SS, Zou X, Zhang X, Lv Y, & Xu J. 2019. Impact of climate change on crops adaptation and strategies to tackle its outcome: A review. *Plants*. 8(2): 34. https://doi.org/10.3390/plants8020034

Robinson S. 2021. Mechanobiology of cell division in plant growth. *New Phytol.* 231(2): 559–564. https://doi.org/10.1111/nph.17369

Sadh PK, Duhan S, & Duhan JS. 2018. Agro-industrial wastes and their utilization using solid state fermentation: a review. *Bioresour. Bioprocess.* 5: 1. https://doi.org/10.1186/s40643-017-0187-z

Sari D & Rahmawati A. 2020. Analisa kandungan limbah cair tempe air rebusan dan rendaman kedelai [Analysis of liquid waste tempe of boiled water and soybean soaking water]. *Jurnal Ilmiah Kesehatan Media Husada.* 9(1): 36–41. https://doi.org/10.33475/jikmh.v9i1.210

Shamkuwar SV, Swarnkar SR, Gupta P, Budhe VK, & Baral SS. 2019. A critical study on weed control techniques. *Int. J. Adv. Agric. Sci.Technol.* 6(12): 1–22.

Siddeeg SM, Tahoon MA, & Rebah FB. 2020. Agro-industrial waste materials and wastewater as growth media for microbial bioflocculants production:

A review. *Mater. Res. Express*. 7(1): 012001. https://doi.org/10.1088/2053-1591/ab5980

Simko I & Piepho H-P. 2012. The area under the disease progress stairs: calculation, advantage, and application. *Phytopathology*. 102(4): 381–389. https://doi.org/10.1094/PHYTO-07-11-0216

Singh UP, Kamboj A, & Sharma M. 2020. Herbicide resistance in weed and its management-a review. *Int. J. All Res. Educ. Sci. Methods.* 8(12): 213–223.

Soesanto L, Mugiastuti E, & Manan A. 2020. The potensial of *Fusarium* sp. and *Chaetomium* sp. as biological control agents of five broad-leaf weeds. *Caraka Tani: Journal of Sustainable Agriculture.* 35(2): 299–307. https://doi.org/10.20961/carakatani.v35i2.35713

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(2): 719–725. https://doi.org/10.13057/biodiv/d220224

Steketee CJ, Martinez-Espinoza AD, Harris-Shultz KR, Henry GM, & Raymer PL. 2016. Effects of genotype and isolate on expression of dollar spot in seashore paspalum. *HortSci.* 51(1): 67–73. https://doi.org/10.21273/HORTSCI.51.1.67

Uikey W, Raghuwanshi KS, & Uikey DW. 2020. Influence of culture media on growth, colony character and sporulation of *Chaetomium globosum* fungus. *Int. J. Curr. Microbiol. App. Sci.* 9(5): 2567–2572. https://doi.org/10.20546/ijcmas.2020.905.293

Ustuner T, Al Sakran L, & Almhemed K. 2020. Effect of herbicides on living organisms in the ecosystem and available alternative control methods. *Int. J. Sci. Res. Publ*. 10(8): 633–641. https://doi.org/10.29322/IJSRP.10.08.2020.p10480

Uysal A & Kurt S. 2017. Influence of inoculum density, temperature, wetness duration, and leaf age on infection and development of spinach anthracnose caused by the fungal pathogen *Colletotrichum spinaciae*. *Eur. J. Plant Pathol.* 149: 1041–1052. https://doi.org/10.1007/s10658-017-1249-y

Seventer JMV & Hochberg NS. 2017. Principles of infectious diseases: Transmission, diagnosis, prevention, and control. In: Quah SR (Ed.). *International Encyclopedia of Public Health (Second Edition*). pp. 22–39. Academic Press. https://doi.org/10.1016/B978-0-12-803678-5.00516-6

Vrabl P, Schinagl CW, Artmann DJ, Heiss B, & Burgstaller W. 2019. Fungal growth in batch culture–What we could benefit if we start looking closer. *Front. Microbiol*. 10: 2391. https://doi.org/10.3389/fmicb.2019.02391

Wongpia A & Lomthaisong K. 2010. Changes in the 2DE protein profiles of chilli pepper (*Capsicum annum* L.) leaves in response to *Fusarium oxysporum* infection. *ScienceAsia.* 36: 259–270. https://doi.org/10.2306/scienceasia1513-1874.2010.36.259

Ziaulhak DY, Soesanto L, & Manan A. 2019. Eksplorasi dan uji virulensi jamur patogen gulma daun sempit di pertanaman tebu (*Saccharum officinaru*m L.) [Exploration and investigation on the virulence of narrow leaf weed pathogenic fungi in the sugarcane plantations]. *Matriks: Jurnal Sosial dan Sains.* 1(1): 18–27. https://doi.org/10.36418/matriks.v1i1.49