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Compost Tea as Biofungicides to Suppress *Sclerotium rolfsii* on Soybean (*Glycine max* L. Merr.)

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Abstract. One of the important diseases that can reduce the productivity of soybean is damping-off disease caused by *Sclerotium rolfsii*. This disease can be controlled by using synthetic pesticides. However, excessive use of pesticides can cause pollution to the environment and the resistance of the pathogen to pesticides. This study aims to determine the effect of compost tea of worm dropping in suppressing the fungus *Sclerotium rolfsii*, the causal agent of damping-off disease of soybeans (*Glycine max* L. Merr). This research was carried out in a glasshouse and Microbiology Laboratory, Faculty of Agriculture, The University of Mataram. The experiments were arranged according to Completely Randomized Design (CRD), which consist of 2 types of compost tea, namely compost tea, which was produced through the aeration process (Aerated Compost Tea-ACT) and produced without aeration process (Non aerated Compost Tea-NCT). The experiment consisted of 7 treatments and replicated 3 times, namely; Control, ACT without dilution, ACT with 10^{-1} dilution, ACT with 10^{-2} dilution, NCT without dilution, NCT with 10^{-1} dilution, and NCT with 10^{-2} dilution. The results showed that in the in vitro experiments both types of compost tea could suppress the growth of the fungus S. *rolfsii*. In the in vivo experiment, the treatment of NCT 10^{-1} dilution and ACT 10^{-1} dilution were categorized as quite effective in reducing the incidence of damping-off disease with the effectiveness rates of 51.72% and 47.13%, respectively

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is one of the most important food crops in Indonesia, after rice and corn. Most of soybean needed by Indonesia is imported. For example, based on BPS data quoted by Bisnis.com [1] that during the first semester of 2020 Indonesia had imported 1.27 million tons of soybean, and suggested to improve the productivity of soybean in Indonesia.

However, there are some constraints in efforts to increase soybean productivity. One important constraints in increasing the productivity of soybean is the attack of diseases such as damping-off disease caused by *Sclerotium rolfsii* [2] This disease can be controlled using chemical pesticides. However, when chemical pesticides were used continuously, they can cause pollution to the agro-ecosystem and the resistance of pathogen to those chemicals. Therefore, it is important to find alternative control that are environmental friendly.

One alternative control that can be done is to use compost tea extract derived from worm droppings compost (vermicompost) which is rich in nutrients and beneficial microorganisms that could be acted as biofungicides. Compost tea is a compost extract that uses water as an extracting agent and an additional food source for microbes such as molasses. There are two methods of making compost tea, namely aerated compost tea (ACT) and non-aerated compost tea (NCT) [3]. Several studies have shown that the use of compost tea can suppress the development of plant pathogens, such as *Fusarium*, *Verticillium*, *Plectosporium*, and *Rhizoctonia* [4]. Therefore, a research had been

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conducted on the effect of compost tea of worm dropping in suppressing the disease caused by the fungus *Sclerotium rolfsii* in soybean.

MATERIALS AND METHODS

This research was conducted on 01 May 2021 to 07 June 2021 at a greenhouse and the Microbiology Laboratory, Faculty of Agriculture, the University of Mataram.

The experiments were arranged according to a completely randomized design (CRD). There were two series of experiments conducted, i.e.: in vitro and in vivo experiments. The in vitro experiments consisted of three treatments including: Control (*Sclerotium rolfsii* was grown on PDA), ACT (*S. rolfsii* was grown on PDA mixed with Aerated compost tea of worm dropping compost), and NCT (*S. rolfsii* was grown on PDA mixed with Non-aerated compost tea of worm dropping compost). Each treatment was repeated six times. While, the in vivo experiments consisted of 7 treatments, namely: control, ACT without dilution (5.2 x 10^6 CFU/ml), ACT 10^{-1} dilution (5.2 x 10^5 CFU/ml), ACT 10^{-2} dilution (5.2 x 10^4 CFU/ml), NCT without dilution (3.8 x 10^6 CFU/ml), NCT 10^{-1}dilution (3.8 x 10^5 CFU/ml), NCT 10^{-2} dilution (3.8 x 10^4 CFU/ml). Each treatment was repeated three times.

Inoculum Preparation of *Sclerotium rolfsii*

Soybean with stem rot symptoms were brought to the laboratory for isolation. The infected part of the stem was cut into small pieces containing healthy and diseased part. The plant parts were then soaked in 70% alcohol solution for 1 minute and then rinsed with sterile distilled water 3 times. The plant parts were then air-dried on sterile filter paper in Laminar Air Flow cabinet, then placed on WA media. The plant parts were incubated at room temperature and then purified on PDA media.

Compost Tea Preparation

Aerated compost tea (ACT) was made by mixing worm dropping compost with clean water at a ratio of 4:1. A container (bucket) containing 1,600 ml of clean tap water was added with 400 g of worm dropping compost and added 2 ml of molasses. The compost tea mixture was stirred and supplied with air (aeration) using an aerator for 3 days. The container is left open and then filtered the compost tea mixture using a cloth filter. The non-aerated compost tea (NCT) was prepared in the same way, but the compost tea was only incubated for 3 days at a room temperature without any aeration process.

Calculation of Microbial Density in Composted Tea

Each compost tea filtrate was diluted using sterile distilled water to a dilution level of 10^{-6}. The suspension at a dilution of 10^{-6} was taken 0.1 ml then placed and leveled on PDA media for fungi and NA media for bacteria using drigalski spatula. Observations were made by counting the microbial colonies that grew on each of these media and the population was counted after 72 hours (3 days).

In vitro Experiment on the Effect of Composted Tea on the Colony Growth of *S. rolfsii*

In vitro experiment was carried out by mixing PDA media with compost tea, with a ratio of 15:100 (compost tea : PDA). Then a piece of sclerotia of *S. rolfsii* were placed in the center of the petri dish. Then calculated the colony growth inhibition of *S. rolfsii* based on the formula by Mori *et al*. [5] as follows and grouped as in Table 1:

$$\mathrm{CGI} = \frac{CDC - CDT}{CDC} x\ 100\%$$

Note:
CGI = Colony growth inhibition

CDC = Colony diameter of control (colony growth in an untreated media)
CDT = Colony diameter of the fungus grown in treated media with compost tea

TABLE 1. *Category of the ability of compost tea to inhibit the colony growth of Sclerotium rolfsii*

Colony growth inhibition (CGI)	Category
CGI > 75	Very strong
75%≤ CGI <50%	Strong
50%≤ CGI <25%	Medium
25%< CGI <0%	Weak
0%	Inactive

In vivo Experiment on the Effect of Composted Tea on *S. rolfsii*

Preparation of Planting Media

The planting medium used was topsoil which was dried in the open air and solarized for 2 weeks and mixed with compost at a ratio of 2:1 (v/v). The media were crushed and sifted, then put into polybags. Each polybag contains 5 kg of planting media.

Soybean Seed Preparation and Planting

Soybean seeds were obtained from Balai Perlindungan Tanaman Pangan (BPTP) Narmada, West Lombok Regency. Soybean seeds were planted in polybags measuring 40 x 40 at a depth of ± 3 cm. Each polybag was planted with 4 soybean seeds. Each treatment and replication used two polybags so that 8 samples of soybean plants were obtained for each treatment.

Compost Tea Application

Application of compost tea was carried out by soaking the seeds for ± 30 minutes and watering once a week by watering at a dose of ± 10 ml per plant. Watering is carried out on the soil in the root area. Each polybag was watered with compost tea at three levels of dilution, namely undiluted, diluted to 10^{-1}, and diluted to 10^{-2}.

Inoculation of S. rolfsii

Sclerotium rolfsii inoculation was done by placing 2 pieces of fungal mycelia with a diameter of 8 mm around the seeds with a depth of ± 3 cm at the same time as planting soybean seeds

Plant Maintenance

Maintenance activities include watering and weeding. Watering is done regularly and periodically every 3 to 4 times a week. Mechanical weeding by removing weeds that grow around the plant.

Parameter Observed

Incubation Period.-- Observation of the incubation period was carried out the day after inoculation of the pathogen until the first symptoms appeared, which were marked by the fall of the sprouts and stem rot.

Disease Incidence.-- Observations of disease incidence were carried out at weeks 1, 2, 3, and 4 after planting. Plants that show symptoms of damping-off disease are then calculated using the formula:

$$DI = \frac{\mathrm{a}}{\mathrm{a} + \mathrm{b}}$$

Note:
DI = Disease Incidence (Disease Intensity)
a = Number of diseased plants with wilting symptoms observed in each polybag
b = Number of healthy plants observed in each polybag

Relative Effectiveness.-- The Relative Effectiveness (RE) of compost tea in suppressing the fungus *S. rolfsii was* calculated using the formula according to Unterstenhofer (1976) in Nurjanani [6] as follows:

$$RE = \frac{\text{DI C} - \text{DI Tr}}{\text{DI C}}$$

Note:
RE = Relative effectiveness
DI C = Disease incidence of control
DI Tr = Disease incidence of treatment

TABLE 2. *Category of relative effectiveness of compost tea in suppressing the disease caused by Sclerotium rolfsii*

Relative Effectiveness (RE)	Category
RE $\geq$ 80%	Very effective
60%$\leq$ RE <80%	Effective
40%$\leq$ CGI <60%	Moderately effective
20%$\leq$ CGI <40%	Less effective
RE < 20%	Not effective

Data Analysis

Data obtained were analyzed using Analysis of Variety (ANOVA) at a 5% significance level. If there is a significantly different in the treatment, then a further test is carried out with the Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Microbial Density in Composted Tea

Population density of microbes in compost tea after being incubated for ±72 hours are presented in Table 3.

TABLE 3. *Population density of microbes in compost tea*

Treatment	Microbes	Population (CFU/ml)
Aerated Compost Tea	Fungi	2×10^6
	Bacteria	3.2×10^6
Non-aerated Compost Tea	Fungi	1.4×10^6
	Bacteria	2.4×10^6

The data showed that the bacterial population is higher than the fungal population. Ingham (3) stated that, in general, in compost tea, the most dominant microbial population is bacteria. The data showed that the microbial density in aerobic compost tea is higher than that in anaerobic compost tea. This is thought to be due to the less than optimum fermentation time of the anaerobic compost tea which only occurs within 3 days. According to Scheuerell [7] the optimum storage period for anaerobic compost tea to allow for an optimal fermentation process is between 7-14 days. Another factor is the absence of a given air supply, as stated by Kelley [8] that the absence of air supply can create anaerobic conditions that can affect the limited growth of microorganisms, especially bacteria. The presence of

air supply can provide aerobic conditions that can support the growth of obligate aerobic bacteria whose growth requires oxygen [9].

In vitro Experiment on the Effect of Composted Tea on the Colony Growth of *S. rolfsii*

The effect of compost tea on the colony growth of *S. rolfsii* in the in vitro experiment showed that each treatment was significantly different than the other treatments (Table 4).

TABLE 4. *Effect of compost tea on the colony growth of S. rolfsii*

Treatment	Colony diameter (cm)	Inhibition	Category
Control	9 [a]	0	
Aerated compost tea	4.7 [b]	51.67	Strong
Non-aerated Compost Tea	1.7 [c]	81.02	Very strong
LSD 5%	0.796		

Note: Numbers followed by the same letter in the same column show no significant difference according to the LSD at the level of 5%.

The highest percentage of inhibition was found in the anaerobic compost tea (NCT) treatment of 81.02% with very strong inhibition category, and followed by the aerobic compost tea (ACT) treatment of 51.67% with strong inhibition category. The two compost tea treatments were higher than the control treatment. This indicates that the microbial activity in compost tea was able to slow down the growth of the fungus *S. rolfsii.* According to Sari [10] that microorganisms in compost tea are able to act as antagonistic microbes that inhibit the growth of pathogens, by competing for space and nutrients needed by pathogens, secreting metabolites (antimicrobials) or becoming parasites for pathogens directly.

The data on the in vitro antagonist test showed that the anaerobic compost tea (NCT) treatment gave a higher suppression of *S. rolfsii* fungus than the aerobic compost tea (ACT). This could be because the application of aerobic compost tea in this study was not applied directly as soon as possible. When the experiment was carried out, the compost tea was allowed to stand for several hours before being applied. According to Dearborn [11], aerobic compost tea will not work optimally if it is not applied directly, because some obligate aerobic bacteria require a constant supply of oxygen.

The effectiveness of compost tea in inhibiting the fungus *S. rolfsii was* not positively correlated with the density of the microbial population. This was because the inhibition of the growth of the fungus *S. rolfsii was* more influenced by the quality of the microbes than the quantity of microbes contained in each compost tea. Based on Koné *et al.* [12] the types of microorganisms that are potential biological control agents contained in compost tea in some cases will be more influential in the suppression effect of pathogens than the high microbial population.

In vivo Experiment on the Effect of Composted Tea on Disease Intensity of *S. rolfsii*

Incubation Period and Disease Intensity

The incubation period for all treatments was the same, namely 3 days for pre-emergence damping-off symptoms and 7-9 days for post-emergence damping-off symptoms. (Table 5).

TABLE 5. *Incubation period and disease intensity*

Treatment	Disease Intensity (%)	Incubation Period (Days)
Control	87.50[a]	3 - 7
ACT without dilution	62.75[ab]	3 – 8
NCT without dilution	54.17[b]	3 – 8
ACT dilution 10^{-2}	50.00[b]	3 – 8
NCT dilution 10^{-2}	50.00[b]	3 – 8
ACT dilution 10^{-1}	45.83[b]	3 – 9
NCT dilution 10^{-1}	41.67[b]	3 – 9

Note: Numbers followed by the same letter in the same column show no significant difference according to Duncan's at the level of 5%.

Analysis of variance showed that the use of aerobic (ACT) and anaerobic (NCT) compost tea had a statistically significant effect on disease intensity. The highest disease intensity occurred in the control treatment. In the ACT treatment without dilution, the disease intensity was not significantly different when compared to the control. Meanwhile, in the treatment of NCT without dilution, ACT with 10^{-2} dilution, NCT with 10^{-2} dilution, ACT with 10^{-1} dilution and NCT with 10^{-1} dilution gave significantly different effects.

The mechanism of suppression of pathogens by these two types of compost tea is thought to occur with competition for space and nutrients the high quantity and diversity of microbes in compost tea. Research by Yasir *et al.* [13] detected 22 microbial strains in vermicompost tea from the genus *Streptomyces spp.*, with antifungal abilities. In addition, other suppression is thought to be through an antibiosis mechanism by bacteria. According to Ingham (3), in aerobic and anaerobic compost tea there are generally PGPB (Plant Growth Promoting Bacteria) from the genus *Pseudomonas* and *Bacillus* which can act as bio-protectants by secreting substances that can inhibit the growth of pathogens.

According to Fardiaz (1982) *in* Nasikhah [14], the ability of an antimicrobial compound to suppress disease is influenced by the level of dilution of the antimicrobial, the more concentrated the antimicrobial substances contained, the higher their antimicrobial activities. However, in this study, it was shown that undiluted compost tea showed higher disease intensity than compost tea with 10^{-2} or 10^{-1} dilutions. This can be caused by the density and interactions between the microbes themselves. Naturally, several types of microbes in the same ecosystem can interact with each other antagonistically [15]. According to Hibbing *et al*. [16], in a community of bacterial species with a dense and diverse population, bacteria compete with other bacteria for space and nutrients. In addition, too many microbial populations are susceptible to nutritional deficiencies if the microbial growth media does not have sufficient nutrients [14]. So that by reducing the population to a certain level through dilution, it is assumed that it can reduce the level of negative interactions on several types of microbes in compost tea.

Relative Effectiveness

Relative Effectiveness (RE) of compost tea in suppressing the fungus S. rolfsii can be seen in Table 6 below.

TABLE 6. *Relative Effectiveness of compost tea in suppressing S. rolfsii*

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Treatment	Relative effectiveness (%)	Category
ACT without dilution	28.73	Less effective
ACT dilution 10^{-1}	47.13	Moderately Effective
ACT dilution 10^{-2}	42.53	Less effective
NCT without dilution	37.93	Less effective
NCT dilution 10^{-1}	51.72	Moderately effective
NCT dilution 10^{-2}	42.52	Less effective

Based on the relative effectiveness of compost tea in controlling *S. rolfsii* in Table 6, the ACT treatment in the 10^{-1} dilution had a relative effectiveness value of 47.13% and the NCT in the 10^{-1} dilution had a relative effectiveness value of 51.72%, both of which were categorize as moderately effective biofungicides which was relatively better than the other treatments. This shows that aerobic and anaerobic compost tea at a dilution level of 10^{-1} can provide a relatively better suppression of disease intensity. This is in accordance with the opinion of Nadiah [17] that the effective application of compost tea is by diluting it first, by mixing 10 parts of water with 1 part of compost tea.

The difference in the ability of compost tea in in-vitro and in-vivo tests to suppress *S. rolfsii* fungus could be due to differences in environmental factors in these two types of experiments. In PDA media there are no external environmental factors that can affect the optimal performance of microbial antagonism in the compost tea solution. The inhibiting factor in the field test is lower than in vitro test due to the fact that after the seeds are planted, the antifungal compounds can decrease due to degradation because they are dissolved and decomposed by environmental factors so that they are not strong enough to work as protectors [18].

CONCLUSIONS

Both types of compost tea (aerated and non-aerated) were able to suppress the growth of *Sclerotium rolfsii* colony with the category of very strong inhibition by the non-aerated compost tea and strong inhibition by the aerated compost tea.

Non-aerated compost tea and aerated compost tea with a dilution of 10^{-1} were categorized as quite effective in suppressing the intensity of damping-off disease caused by *S. rolfsii* with the relative effectiveness of 51.72% and 47.13%, respectively.

REFERENCES

[1] Bisnis.com, retrieved from https://ekonomi.bisnis.com/read/20210105/12/1338923/kedelai-langka-pemerintah-harus-fokus-pada-peningkatan-produktivitas (2021).
[2] I. R. Sastrahidayat, S. Djauhari, N. Saleh and A Muhibuddin, AGRIVITA **33** (1), 40-46 (2011).
[3] E.R. Ingham, *The Compost Tea Brewing Manual, 5th Edition*. (Soil Food Web, Inc., Corvallis, 2005).
[4] C.A. Edwards, N.Q. Arancon, and S. Greytak, BioCycle **47** (5) retrieved from https://www.biocycle.net/effects-of-vermicompost-teas-on-plant-growth-and-disease/ (2006), p. 28.
[5] M. Mori, M. Aoyama, S. Doi, A. Kanetoshi and T. Hatashi, Holz als Rohund Werkstoff **55**, 130-132 (1997).
[6] Nurjanani., *Kajian Pengendalian Penyakit Layu Bakteri (Ralstonia solanacearum) Menggunakan Agens Hayati Pada Tanaman Tomat*, (Balai Pengkajian Teknologi Pertanian Sulawesi Selatan, Makassar) retrieved from https://www.slideshare.net/xie_yeuw_jack/4-nurjananiagens-biokontrol (2011).
[7] S.J. Scheuerell and W.F. Mahaffee, Phythopathology, **94**, 1156 – 1163 (2004).
[8] S. Kelley, BioCycle. 45(6), retrieved from https://www.biocycle.net/building-a-knowledge-base-for-compost-tea/ (2004), p. 32.
[9] R.J. Kadner and K. Rogers, Bacteria "Encyclopedia Britannica", retrieved from bacteria - Physical requirements | Britannica
[10] S. Sari, "Pengaruh Penggunaan Teh Kompos Untuk Menekan Perkembangan Penyakit Hawar Daun (Pantoea sp.) pada Tanaman Jagung (Zea mays L.)," Thesis, Universitas Brawijaya (2013).
[11] Y. Dearborn, Compost Tea: Literature Review on Production, Application and Plant Disease Management, retrieved from https://sfenvironment.org/sites/default/files/editor-uploads/toxics/pdf/sfe_th_compost_tea_review_6.17.11_final.pdf (2011).
[12] S. B. Koné, A. Dionne, R.J. Tweddell, H. Antoun, and T. J. Avis, Journal of Biological Control, **52**, 167-173 (2010).
[13] M. Yasir, Z. Aslam, S. W. Kim, S. W. Lee, S.W., C. O. Jeon, and Y.R. Chung, Bioresour Technol., **100** (19), 4396–4403.
[14] K. Nasikhah, "Pengaruh Isolat Alami *Pseudomonas fluorescens* Pada Beberapa Tingkat Pengenceran Terhadap Jamur *S. rolsfii* Penyebab Penyakit Layu Pada Kedelai (*Glycine max* (L) Merill)" Undergraduate Thesis, Universitas Islam Negeri Malang, 2008.
[15] M.R. Rifa'i, H. Widowati, and A. Sutanto, Biolova, **1** (1). 21-26
[16] M. E. Hibbing, C. Fuqua, M. R. Parsek, and S. B. Peterson, Nat Rev Microbiol. **8** (1), 15–25 (2010)

[17] A. Nadiah, *Petujuk Teknis "Pengembangan Teknologi Teh Kompos Untuk Pengendalian OPT Perkebunan"* (Balai Besar Perbenihan dan Proteksi Tanaman Perkebunan Surabaya, 2012).
[18] B. A. K. Khaerullita, "Pengaruh Beberapa Ekstrak Nabati Terhadap Penyakit Rebah Kecambah Oleh *Sclerotium rolfsii* Sacc. Pada Tanaman Kedelai," Undergraduate thesis, the University of Mataram, 2017

CERTIFICATE

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This is to certify that

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