Effectiveness of fermentation extract of cabbage leaves from harvesting waste to control purple blotch of garlic

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Abstract. Purple blotch caused by *Alternaria porri*, has an impact on garlic plantings. Furthermore, the severity of this disease on local garlic varieties can be greater than 60%. This led to the use of a biological control involving agricultural waste biomass of cabbage leaves as a form of alternative control against this disease. Also, these biological waste biomasses were chosen because their fermentation process produces antimicrobial compounds, which inhibit pathogen growth. This research aimed to analyse the effectiveness of fermentation extract of cabbage leaves, in order to control purple blotch on garlic, the level of concentration needed, and the frequency of the most effective applications.

Furthermore, it used a completely randomized design for in vitro testing, and the non-factorial randomized complete block model for in vivo analysis, which involved 8 treatments. The results showed that the fermentation extract with a concentration of 200 mL.L\(^{-1}\), effectively inhibited 100% of colony growth, while the bi-weekly application of this product at 300 mL.L\(^{-1}\), effectively suppressed the disease incidence of purple blotch, by up to 74.69%.

Keywords: *Allium*, *Alternaria porri*, biopesticides, plant disease, metabolite compounds

1. Introduction
Garlic (*Allium sativum* L.) is an economically important commodity, with B/C ratio of farming 1.28 [1], which is highly demanded because of its service as both food flavoring agent and traditional medicine [2]. This has caused a high level of demand for this commodity, which unfortunately unsatisfying by the national production. Garlic production in 2016 only amounted to 21.151 tons, while it decreased to 19.510 tons in 2017 [3]. Additionally, this decrease was caused by pests and diseases, with purple blotch caused by *Alternaria porri* Ell. Cif. having an impact on garlic plantings. Furthermore, its severity on local garlic varieties from fields in Sembalun and Santong, Lombok, is greater than 60% [4], at range of 50-90% [1], while also occurring over 60% in onion [5].

Also, the non-selective use of chemical pesticides, causes plant-disturbing organisms to become resistant, destroys their natural enemies [6], while posing a threat to both the environment and human
health. Therefore, new strategies are being developed continuously, in order to reduce dependence on synthetic agrochemical [7]. This research offers a solution, where extra agronomic wastes are used as bio-pesticides. Cabbage leaves are agricultural wastes which when left unprocessed, causes environmental pollution, therefore, getting them fermented. Also, the organic acids produced by microbial agents of fermentation are known to produce antimicrobial compounds, which inhibits the growth of pathogen [8].

This research aims to study the effectiveness of cabbage leaf fermentation extract (FECL), in controlling the purple blotch of garlic, while studying the required concentration and the frequency of the most effective application.

2. Materials and methods

In this study, 250 g of cabbage leaves, 500 mL each of water, and cow urine, with 100 g of sugar, were fermented for 28 days. Afterwards, the fermentation products were filtered with a tea strainer, accompanied by a cotton cloth. Also, In vitro testing used a completely randomized design (CRD), with 3 replications. Furthermore, EFDK of concentrations 0, 50, 0, 200 mL.L⁻¹; and Mancozeb 800 ppm were mixed with potato sucrose agar (PSA), using the Poisoned Food method. The percentage inhibition of the mycelial growth was then calculated, using the formula as described by Vincent [9], as follows,

\[ I(\%) = \frac{C - T}{C} \times 100 \]

Where, I= percentage of inhibition of mycelial growth, C= radial growth of fungus in control, and T= radial growth of fungus in treatment.

The inhibitive activity of the fermentation extract was also tested in vivo, at a plastic house in Pancot, Tawangamangu. This test used a non-factorial randomized complete block design, consisting of 8 treatments, in combination with the concentration of FECL and frequent application, with three replications. Furthermore, it consisted of: no treatment, Mancozeb 800 ppm, FECL 150 mL.L⁻¹ and once a week application, FECL 150 mL.L⁻¹ and bi-weekly application, FECL 200 mL.L⁻¹ and once a week application, FECL 200 mL.L⁻¹ and bi-weekly application, FECL 300 mL.L⁻¹ and once a week application, and finally FECL 300 mL.L⁻¹ and bi-weekly application.

Three clove seeds were planted in a polybag at depths of 2-3 cm in the soil, and 10 cm from one another. Moreover, the growing media in each polybag contained 2 kg of soil and cow manure in the ratio 1:1, with A. porri inoculated at day 70 of planting. Also, suspensions of this fungi with concentration of 10⁶ spores.mL⁻¹ dissolved in 100mL sterile water, were sprayed on the surfaces of injured leaves, by using a cutter. The FECL were also applied a week before the inoculation, while the dosage of the extract solution used was 25mL.polybag⁻¹. However, observations were conducted on the number and area of lesions (cm²), disease incidence (%), plant height (cm), and amount of leaves.

Furthermore, garlic were harvested 120 days after planting, and the variables (plant wet weight (g) & bulb weight (g)) were observed. The collected data on the different parameters were then statistically analyzed by the analysis of variance (ANOVA), accompanied by Duncan’s Multiple Range Test (DMRT) at 5% level of significance, using the SPSS program.

3. Result and discussion

3.1. Fermentation of cabbage leaves

The fermentation of the cabbage leaves was carried out for 28 days, with the colour change observed in the extract (FECL) being a sign of a successful process. The fresh green colour had turned to brownish-yellow, which resulted in a yellowish-green residual precipitate from the decomposition of the leaf biomass. Also, organic materials present in the cabbage leaves were destroyed by microorganisms, through an enzymatic process.
Furthermore, the basic ingredient of the FECL was a mixture of both the cabbage leaves and cow urine, as source of bio-starter and nitrogen. Also, cabbage is an herbaceous plant, rich in polysaccharides, and also having a high-water content of 92.44% [10], while fresh cow urine contains 36.90-37.31% nitrogen [11], which unites with microbes during the decomposition of organic material [12]. Both of these ingredients provided nutrients that were very beneficial for the activity of the microorganisms, with the decomposer requiring an energy source (C and N) for protein synthesis [13]. Suprihatin and Perwitasari [14], stated that the decomposed cabbage waste became a location for fermentation microbes, such as Lactobacillus vivorum, L. delbruki, L. fermentum, and L. brevis.

Moreover, the fermentation process showed a C-organic content of 1.13% and a total N of 0.53%, resulting in a C/N ratio of 2.12. Also, the mass of the liquid tended to be higher than the solid, resulting in low C-organic levels. A low level of N was also observed in the fermentation extract, which was influenced by the release of ammonia. Furthermore, Irpan et al. [15], stated that the nitrogen content produced in the fermentation process was released into the air in the form of NH3 gas. In accordance with this, the nitrogen present in the cow urine was in the form of ammonia gas, and was collected in a closed container. Therefore, there was need for it to be released, in order to prevent an explosion. Also, Rizki et al. [16], stated that the nitrogen in cow urine was in the form of ammonia compounds, which possesses high temperatures.

The initial fermentation temperature was 30°C, which after a brief period dropped to 28.5°C. This gradual drop in temperature occurred because the fermentation process was continued by mesophilic microbial activity. Also, Suwatanti and Widiyaningrum [17], stated that the temperature gradually decreased, due to the reduced organic material available for decomposition by microorganisms. Panda et al. [18], also added that the degradation of fruit and vegetable waste was carried out by several microbes, such as Lactobacillus sp., Aspergillus niger, Saccharomyces cerevisiae, and Acetobacter aceti, and produced organic compounds in the form of lactic, citric, and acetic acids. However, the initial pH of the fermentation process at 7 gradually became 4, due to the presence of microbial activity in producing organic acids.

3.2. Inhibitory activity of cabbage leaves fermentation extract on the growth of Alternaria porri colonies
The application of FECL at a concentration of 200mL.L−1, effectively inhibited the growth of A. porri colonies by 100% (Figure 1). This was confirmed by the absence of fungal colony growth in the Petri dishes (Figure 2). Also, A. porri colonies were shown in grayish-black with a black reserve colony [19]. Furthermore, the research of Rakesh et al. [20] showed that fermented cow urine extract in combination with Artocarpus lakoocha, Hemedesmus indicus, Croton roxburghi, and Maesa indica plants, effectively inhibited the pathogen, Fusarium oxysporum.

Also, Mancozeb 800 ppm had the same effectiveness as this extract, at a concentration of 200mL.L−1. Madhavi et al. [21], also showed that Mancozeb had a high effectiveness in inhibiting the growth of A. porri colonies, with the research of Rahman et al. [22] further showing its antimicrobial activity on Lactobacillus vivorum, Lactobacillus brevis, and Pediococcus pentosaceus, which were isolated from the fermentation of vegetable waste.

The antifungal mixture in FECL was formed, due to its microorganism activity. Damayanti et al. [23], also stated that its antifungal composition, which was a product of extracellular metabolites, was produced during the growth process of lactic acid bacteria in the fermentation medium.
3.3. Effectiveness of cabbage leaves fermentation extract from harvesting waste to control purple blotch

FECL had the potentials to be an environmentally friendly alternative biological control of purple garlic spots. Its effectiveness in suppressing the development of purple blotch disease (Table 1), was due to the presence of antimicrobial compounds, which occurred as a result of microorganism activity. Microorganisms and compounds resulting from metabolic activities in FECL, acted as scavenger agents in increasing plant resistance. Additionally, Inayati [24] stated that these agents/elicitors work, by stimulating and activating resilience responses from within plants. Therefore, the application of FECL seemed to have been able to influence plant resistance to pathogen infections.

Bi-weekly application of the FECL at a concentration of 300 mL.L⁻¹, effectively suppressed the development of the disease. Moreover, applications at this concentration seemed to have succeeded in activating a resistance response from within the plant, through the epidermis of its leaves. Similar results were shown by the application of FECL at a concentration 150 mL.L⁻¹ with spray, once a week. The microorganisms in the FECL colonized the plant tissue, preventing the pathogens from being in control. Also, pathogens require sufficient energy in the process of infection and colonization [28]. The microorganisms in the FECL competed seriously with the pathogens for energy, in order to ensure the survivability of the plants against the pathogenic attack.

| Concentration (mL.L⁻¹) | Frequency (x.week) | Disease Incidence (%) | Effectiveness of Disease Suppression (%) |
|------------------------|--------------------|-----------------------|----------------------------------------|
| 150                    | 1                  | 0.04±0.42 abc         | 69.69±18.45 b                          |
|                        | 2                  | 17.24±3.48 c          | 03.89±29.00 a                          |
| 200                    | 1                  | 0.00±6.41 abc         | 55.57±41.41 ab                         |
|                        | 2                  | 0.53±5.46 ab          | 68.92±47.75 ab                         |
| 300                    | 1                  | 15.16±6.21 bc         | 52.07±34.44 ab                         |
|                        | 2                  | 0.33±6.56 ab          | 74.69±24.56 b                         |
| Mancozeb 800ppm        |                    | 0.11±1.92 a           | 99.92±00.14 b                         |
| No Treatment           |                    | 17.37±0.56 c          | -                                      |

The values represent mean±Sdv. and the numbers in the same column, followed by the same letters are not significantly different from Duncan’s multiple range test of 5% level (P<0.05).
Malinovsky et al. [25], stated that pathogens have challenges in penetrating the physical barrier of host plants, while avoiding detection by their resistance receptors. Also, a positive response from the receptors was indicated by the low rate of disease incidence, as the application of FECL was effective in reducing the rate of abnormal incidence (Table 1). Also, the Mancozeb fungicide had a higher ability in controlling purple blotch, compared to FECL. Putri et al. [26], also stated that Mancozeb killed pathogens by forming a thin layer on the surface of plants.

Malinovsky et al. [27] stated that changes in plant cell walls were triggered, due to attack responses caused by pathogens. Also, these positive responses eventually caused symptoms. Moreover, purple blotch was indicated by the presence of small white concave patches which turns brown, forming an enlarged concave zone and purplish colour [28]. All visible symptoms were measured as a result of the infection process, and success was indicated by the number of spots formed. Also, the application of FECL was effective in reducing the developments of symptoms, which was indicated by the lower number and extent of lesions (Table 2).

**Table 2.** Effectiveness of cabbage leaves fermentation extract on the progress of purple blotch symptoms

| Concentration (mL.L⁻¹) | Frequency (week) | Number of Lesion | Area of Lesion (cm²) |
|------------------------|-----------------|-----------------|---------------------|
| 150                    | 1               | 2.00±0.00 abc   | 0.21±0.12 a         |
|                        | 2               | 4.67±1.53 cd    | 0.69±0.20 b         |
| 200                    | 1               | 1.33±1.53 ab    | 0.33±0.32 ab        |
|                        | 2               | 1.67±1.53 abc   | 0.21±0.33 a         |
| 300                    | 1               | 4.00±2.65 bcd   | 0.36±0.29 ab        |
|                        | 2               | 1.67±2.08 abc   | 0.18±0.17 a         |
| Mancozeb 800ppm        | 0.33±0.58 a     | 0.00±0.00 b     |
| No Treatment           | 5.33±0.58 d     | 0.72±0.06 a     |

The values represent mean±Sdv. and the numbers in the same column, followed by the same letters are not significantly different from Duncan's multiple range test of 5% level (P<0.05).

Disease was unable to develop during this period, therefore severity was quite low. Also, this low severity was indicated by less symptoms on the leaves, therefore signifying that photosynthate translocation of the plant was not affected (Table 3).

**Table 3.** Effect of cabbage leaves fermentation extract on the growth and production of garlic

| Concentration (mL.L⁻¹) | Frequency (week) | Plant Height (cm) | Number of Leaf | Wet Plant Weight (g) | Bulb Weight (g) |
|------------------------|-----------------|------------------|----------------|----------------------|-----------------|
| 150                    | 1               | 48.63±9.49a      | 4.56±1.34a     | 68.54±42.11a         | 41.51±22.19a    |
|                        | 2               | 48.73±5.69a      | 4.39±1.00a     | 55.72±13.38a         | 35.17±10.58a    |
| 200                    | 1               | 53.80±2.13a      | 4.29±0.36a     | 85.47±32.90a         | 51.83±18.71a    |
|                        | 2               | 50.24±8.17a      | 4.38±0.70a     | 74.76±53.25a         | 40.50±28.35a    |
| 300                    | 1               | 46.91±5.64a      | 4.28±1.07a     | 67.75±44.50a         | 40.34±24.58a    |
|                        | 2               | 47.12±9.48a      | 3.83±1.61a     | 58.51±55.80a         | 38.36±34.53a    |
| Mancozeb 800 ppm       | 45.77±4.67a     | 3.83±0.17a       | 70.61±30.60a    | 38.06±16.81a         |                |
| No Treatment           | 46.16±9.64a     | 5.39±0.35a       | 79.41±35.91a    | 46.19±21.19a         |

The values represent mean±Sdv. and the numbers in the same column, followed by the same letters are not significantly different from Duncan's multiple range test of 5% level (P<0.05).
4. Conclusion
Fermentation extracts of cabbage leaves from harvesting wastes, effectively suppressed the development of purple blotch by 74.69%. Also, this extract was more effective and efficient when applied bi-weekly at a concentration of 300 mL.L⁻¹.

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