The effectively test of the metalaxyl on different concentrations and application intervals to Phytophthora infestans that caused potato late blight in the wet season in the Karo highlands

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Abstract. Potato (Solanum tuberosum) is short-lived annual crops of high economic value tubers. In the cultivation of potato plants there are many problems faced by farmers. One of which is potato leaf blight caused by the fungus Phytophthora infestans is one of important pathogen in potato crop. This research was conducted in the Plant Disease Laboratory, Faculty of Agriculture Universitas Sumatera Utara and in Karo District of North Sumatra (wet season). Two tests were done, in vitro and in the field, respectively. In vitro test used a non factorial Complete Randomized Design with active ingredients concentration of Metalaxyl 50ppm, 75ppm and 100ppm and in the field was done with factorial Randomized Block Design with 2 factors: the first factor was the concentration level of fungicide 0g/l, 2g/l, 3g/l, 4g/l and the second factor was spraying intervals of 4 days, 8 days and 12 days. The results showed that the in vitro test at a concentration of 100 ppm could inhibit fungal growth by 22.98% and the metalaxyl field test and application interval ineffective to inhibit P. infestans fungal infection and the damage reached 100% in control trials at 66 days after planting.

1. Introduction
Potato plant (Solanum tuberosum) is a short-lived seasonal crop. Potatoes are root crops with high economic value and provide more benefits for farmers because the price of tubers is relatively stable and potato tubers can be kept longer than other vegetables [1]. In potato cultivation, many factors cause tuber production to decrease, one of them is a leaf blight which can cause yield losses of up to 100% [2].

Potato leaf blight is caused by Phytophthora infestans which is attacked and will show symptoms of slightly wet spots, brown to black, with white leaf edges which are sporangium and the leaves will rot or die [3]. Leaf spots can develop on the petiole and stem, which expand with a long shape. When attacking the tubers, the skin color of the infected potato tubers looks faded irregularly, curves and is slightly watery. When cleaved, the potato tuber flesh will appear brown [4].

Potato blight has long been a problem for potato farmers and is the most serious disease among the diseases affecting potato crops in Indonesia [5]. In the Karo highland this disease has spread widely in three districts in Berastagi, Kabanjahe and Merek, were known that this disease is endemic to infect farmers’ potato planting centers. It is known that every 6 location points in 1 sub-district of potato cropping have been infected by P. infestans [6].
The control of late blight *P. infestans* has been carried out by spraying fungicides. The use of fungicides is one method that is still widely used today [7]. Farmers' habit of spraying pesticides freely led to the emergence of new strains of pathogenic fungi by spraying synthetic fungicides at twice the recommended concentration [8]. Based on this, researchers researched to find out several concentration levels and differences in the application interval of systemic fungicide with active ingredient Metalaxyl against *P. infestans* which causes potato blight.

2. **Materials and methods**

2.1. **Research methods**
This research was conducted at the Laboratory of Plant Diseases Faculty Agricultural Universitas Sumatera Utara and in Berastagi, Karo, Sumatera Utara Province. The research laboratory used *P. infestans* isolated from infected leaves from potato fields, purified and grown in V8 juice as a growing medium, the fungicide metalaxyl. In the field using Granola potato seeds (G2). The research parameter was the inhibition test observed when the *P. infestans* was isolated and cultured on V8 juice media which had been treated and observed *P. infestans* fungal growth with 5 experimental replications, disease incidence and disease severity were observed when the plant was in planting field.

2.2. **Isolate fungi from plant parts**
Direct isolation from infected leaves. Leaves infected with *P. infestans* were taken from random potato fields in the field, wrapped in a tissue and put in a plastic bag. Then the infected leaf sample was cut into 5 mm size, sterilized by immersing in 5% sodium hypochlorite (NaOCl) solution for 5 minutes, then washed twice with sterile distilled water, dried with sterile filter paper and then placed on V8 media. and incubated in the dark at room temperature for 1 week. The growing fungal colonies were then observed and purified into single colonies [9].

2.3. **V8 juice media**
The media used is V8 (Vegetable juice), which is 200 ml V8 mixed into agar that has been cooked with 800 ml of water then added 2 grams of CaCO3 and mixed until the medium is homogeneous. When finished, the V8 is poured into a 500 ml Erlenmeyer flask, then covered with cotton and aluminum foil. Sterilize for 15 minutes at 121°C. After the Erlenmeyer temperature has cooled down or has reached a temperature of 45°C, the V8 medium is poured into a petri dish that has been previously sterilized [10].

2.4. **Fungicide application in the laboratory**
Fungicide application of the active ingredient is carried out by making a fungicide stock solution and then adjusting it for each treatment.

2.5 **In vitro experiment**
Laboratory trials using a non-factorial completely randomized design, with a factor of differences in the concentration of the metalaxyl fungicide
D0 = control
D1 = 50ppm of metalaxyl fungicide 25WP
D2 = 75ppm of metalaxyl fungicide 25WP
D3 = 100ppm of metalaxyl fungicide 25WP

2.6. **Fungicide application in the field**
The fungicide with the active ingredient Metalaxyl is applied when the plant starts to see black spots or shows symptoms and is used to prevent the occurrence of a more severe attack of the disease that
will attack the potato crop which is given 12 applications for the treatment interval of 4 days, 8 times the application for treatment interval of 8 days, and 4 applications for 12 days interval treatment.

2.7. In the field experiment
Field trials used a factorial randomized block design, with the first factor the concentration of the fungicide metalaxyl and the second factor the application interval, the trials factor:
The first factor:
D0: No fungicide
D1: Metalaxyl fungicide with a concentration of 2.0 gr / l water
D2: Metalaxyl fungicide with a concentration of 3.0 gr / l water
D3: Metalaxyl fungicide with a concentration of 4.0 g / l of water
[11].
The second factor:
I1: once every 4 days
I2: once every 8 days
I3: once every 12 days
Calculated the disease severity used scoring and formula:
The scoring:
0 = No damage at all
1 = Area of damage to crops> 0 - ≤ 10%
2 = Area of damage to crops> 10 - ≤ 20%
3 = Area of damage to crops> 20 - ≤ 40%
4 = Area of damage to crops> 40 - ≤ 60%
5 = Area of damage to crops> 60% - dead
[12].
The formula:
\[ K_{jp} = \sum \frac{n_i v_i}{Z N} x 100\% \] (1)
In which:
\( K_{jp} \) : Disease severity
\( n_i \) : Total plant tissue attacked in each category
\( v_i \) : Category attack
\( Z \) : Highest attack category
\( N \) : Total of the observed network
[13].

2.8. Statistical analysis
If the results of the analysis of variance show a significant effect, then proceed with the Mean Difference Test based on the Duncan Multiple Range Test (DMRT) at the 5% level [14].

3. Results and discussion
3.1. Inhibition test
Inhibition was observed for 8 days after incubation in the V8 juice medium which had been treated showed the highest inhibitory power results with a value of 22.89% at 100ppm treatment, the content of the active ingredient in V8 juice media and the lowest inhibitory power results in the control treatment with a value of 0 % can be seen in (table 1) as follows:
Table 1. Observation of Inhibition of Phytophthora infestans colony.

| Metalaxyl Treatment | Inhibition after 8 days of incubation (%) |
|---------------------|------------------------------------------|
| 0 ppm               | 0.00a                                    |
| 50 ppm              | 16.00b                                   |
| 75 ppm              | 18.00b                                   |
| 100 ppm             | 22.89c                                   |
| Average             | 14.22                                    |

Based on (table 1) observations of the inhibition test of fungicides in the laboratory did show significant results in inhibiting the growth of *P. infestans* in the 100ppm treatment with other treatments showed significant results on the inhibition of *P. infestans* colonies. The data in Table 1 the inhibition test of Metalaxyl fungicide is not more than 50% which is not very significant to the inhibition of pathogens. This is because the pathogen still has a sensitivity to fungicides, although the inhibition test is not more than 50%. Metalaxyl fungicide is still able to inhibit the development of pathogens in vitro. This is consistent with the research of Wachjadi et al. [15] which states that fungicide treatment shows better results in suppressing the development and rate of infection of leaf blight. Metalaxyl fungicide treatment can inhibit the development of pathogens *P. infestans*. According to Davidse et al [16] the fungicide metalaxyl which belongs to the Phenylamide group was introduced against oomycetes, is highly effective for late blight management and is in great demand worldwide. A specific systemic fungicide, mefenoxam (the active isomer in metalaxyl) inhibits sporulation and mycelial growth in the host tissue by specifically inhibiting the formation of RNA polymerase-1 [17,18].

3.2. Disease incidence

Table 2. Disease incidence (%).

| Metalaxyl treatments | 4 days | 8 days | 12 days | Average |
|----------------------|--------|--------|---------|---------|
| 26 days after planted|        |        |         |         |
| 0 gr/l               | 88.89  | 88.89  | 66.66   | 81.48a  |
| 2 gr/l               | 55.55  | 55.55  | 55.55   | 55.55a  |
| 3 gr/l               | 11.11  | 22.22  | 55.55   | 29.63b  |
| 4 gr/l               | 44.44  | 77.78  | 22.22   | 48.15b  |
| Average              | 50.00  | 61.11  | 50.00   | 53.70   |
| 30 days after planted|        |        |         |         |
| 0 gr/l               | 100.00 | 100.00 | 100.00  | 100.00  |
| 2 gr/l               | 88.89  | 88.89  | 100.00  | 92.59   |
| 3 gr/l               | 88.89  | 77.77  | 88.89   | 85.18   |
| 4 gr/l               | 66.66  | 88.89  | 88.89   | 81.48   |
| Average              | 86.11  | 88.89  | 94.44   | 89.81   |
| 34 days after planted|        |        |         |         |
| 0 gr/l               | 100.00 | 100.00 | 100.00  | 100.00  |
| 2 gr/l               | 100.00 | 100.00 | 100.00  | 100.00  |
| 3 gr/l               | 100.00 | 100.00 | 100.00  | 100.00  |
| 4 gr/l               | 100.00 | 100.00 | 100.00  | 100.00  |
| Average              | 100.00 | 100.00 | 100.00  | 100.00  |
Based on the observation of disease incidence, that the plants in the field 34 days after planting were infected with \textit{P. infestans} as a whole with a value of 100\% and can be seen in the (table 2).

Based on table 2 showed the treatment of fungicide concentrations and application intervals did not significant difference in the disease incidence, seen at 26 days after planting the incidence of the disease was still low, but at 34 days after planting the incidence rate of the disease reached 100\% this was due to rainfall and humidity high in the experimental field that supports the development of \textit{P. infestans}, this is according to [19] Spores will sprout when the air is humid and dewy. At a temperature of 18-21°C disease develops rapidly, especially with the support of a humid environment, and according to [20] leaf blight multiplies very quickly in high rainfall, high humidity and low temperatures.

3.3. Disease severity
The level of the disease severity is calculated based on a scoring scale according to the results of observations of the value of the disease severity at 66 days after the highest plant in treatment D0 the average result reaches 100\% and the lowest severity level is in D3 treatment with an average value of 95.25\% can be seen in (table 3) as follows:

This is because Potato plants that were not applied with fungicides tended to have a higher percentage of the disease severity than the treatment applied the fungicide metalaxyl which has a systemic method of controlling disease. This is consistent with the study of [21] which stated that systemic fungicides are more durable than protective (contact) fungicides used to control late blight. Systemic fungicides can reach the deeper parts of the plant and they remain active deeper in the plant tissue.

Disease severity without fungicide treatment showed the highest results for the disease severity. Plants that have been infected with fungi and are not given prevention as the application of the fungicide Metalaxyl will result in high infestation and widespread. According to [22,23] using fungicides is the most effective measure and will provide one of the best long-term solutions in controlling blight. Metalaxyl and oxadixyl are systemic fungicides that properly control the disease as well as protective activity against oomycetes pathogens.

\textbf{Table 3. Disease severity after 66 planting days (\%).}

| Treatment | Application interval | Average |
|-----------|----------------------|---------|
| Metalaxyl |                      |         |
| 0 gr / l (D0) | 4 days (I1) | 100.00  |
|           | 8 results (I2)    | 100.00  |
|           | 12 days (I3)      | 100.00  |
|           | Average           | 100.00  |
| 2 gr / l (D1) | 4 days (I1) | 88.79   |
|           | 8 results (I2)    | 100.00  |
|           | 12 days (I3)      | 100.00  |
|           | Average           | 96.26   |
| 3 gr / l (D2) | 4 days (I1) | 90.89   |
|           | 8 results (I2)    | 96.54   |
|           | 12 days (I3)      | 100.00  |
|           | Average           | 95.81   |
| 4 gr / l (D3) | 4 days (I1) | 95.34   |
|           | 8 results (I2)    | 94.10   |
|           | 12 days (I3)      | 96.32   |
|           | Average           | 95.25   |
|           | Average           | 93.76   |
|           | Average           | 97.66   |
|           | Average           | 99.08   |
|           | Average           | 96.83   |

The severity of the disease is influenced by climatic conditions, one of which is humidity. At high humidity, the air density will be higher which can prevent fungicides from getting into the potato plant leaves. According to Budi [24] at a humidity of more than 80\%, the air condition is easier to hydrolyse the pesticide spray grain so that it can reduce its toxicity. Air droplets in the form of fog block the speed of the spray grain from reaching the spray field or the plant surface.

4. Conclusions
Inhibition test of \textit{P. infestans} using the fungicide metalaxyl on 8 days of observation showed that were able to inhibit a result of 22.89\%. Fungicide concentration and interval fungicide application in the wet season showed a high disease incidence with damage values reaching 100\% at 34 days after planting and disease severity of the disease with damage values reaching 100\% at 66 days after planting.
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