Response of tomato (*Lycopersicon esculentum* Mill) infected by *Phytophthora infestans* (Mont.) De Bary with the treatment of copper and potassium phosphate on plant development

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Abstract. *Phytophthora infestans* is an important disease in the highland of Karo Regency, North Sumatera, Indonesia. The disease caused a failure of tomato production. *P. infestans* mainly damages the leaves causing disruption to plant growth and development. This study was to evaluate the development of tomato plants infected by *P. infestans* treated with potassium phosphite and copper. The experimental was set in a completely random design, consisted of two factors. The first factor was copper with 2 doses namely 0 %, 0.07 % and the second was potassium phosphate with 4 level doses each 0%, 0.4 %, 0.8 % and 1.2 %. The result showed that potassium phosphate could significantly reduce disease severity of *P. infestans* by 31.62 % to 36.40 %, 40.26 to 63.65 % at dose of 0.4 % and 0.8 % respectively. Tomato infected by *P. infestans* showed a positive response to copper and potassium phosphate treatments which characterized by an increase in the plant dry weight, the plant height and the plant stem diameter. The best dry weight of plant was obtained from Cu at dose of 0.07 % and potassium phosphate at dose of 1.2 % while the plant height was obtained from Cu at dose of 0.07 % and potassium phosphate at dose of 0.4 %.

1. Introduction
Late blight caused by *Phytophthora infestans* (Mont.) De Bary is the most important disease occurs in highland regions including in Karo Regency, North Sumatera, Indonesia. The disease causes devastating production on tomato [1-2]. The temperature in Karo was 19ºC on average and 16.5ºC on minimum, humidity was 88% [3] This climate was a favourable condition for the growth of tomato plants and *P. infestans* as well [4], therefore the intensity of *P. infestans* attacks was quite high. The control efforts carried out by farmers so far have been the intensive use of fungicides especially in wet season. [5-6] suggested that control diseases by using fungicides has led to increased environmental quality concerns, health problems and resistance therefore it is necessary to look for environmentally friendly technology control components. [7] Stated that potassium phosphate had low impact on
environment and it was able to be used as a fungicide to control *P. infestans* which help in the sustainable management practices. The study of copper which could minimize the disease severity caused by many species of plants pathogens had been reported. [8] Investigated the effects of copper (Cu) on wheat plant’s soil naturally infected by *Gaeumannomyces gamines* [Sac] and reported that Cu-deficient plants were more susceptible to pathogen than the plants without Cu deficiency. [9] Suggested that 50 ppm copper could reduce the severity of *Ganoderma boninense* disease in oil palm plants to 15.8% while control was 58.3%.

The objective of this research was to evaluate the response of tomato plants infected by *P. infestans* with the treatment of copper and potassium phosphate in a greenhouse on plant development.

2. Material and Methods

2.1. Research site
The study was conducted in Berastagi, Karo District, located at coordinate 2º50'-3º19' North Latitude, 97º55'- 98º38' East Longitude with an altitude of 1300 meter above sea level.

2.2. Preparation of planting media and plants
Thirty-days F1 hybrid tomato cv. Intern plants were moved to the green house and transplanted to the 24 litter polyethylene pot containing 21 litter of sterilized Andisol soil and 0.48 litter of sterilized cow manure, (C/N 12.10%, N 0.41%, P 14.96%, K 0.10%, Ca 3.85%, Cu 30.78 ppm and pH 6.20). Fertilizer used was double in accordance to [10]. Plants were organized with the spacing of 80 cm between pots and 150 cm between rows. They were 2 main stems each plant. The growing new side shoots were removed.

2.3. Potassium phosphate and copper application
Potassium phosphate 54.5% with a phosphorous acid content of 34.30 % (Luxembourg-Pamol,Inc.) and copper source as E-Cu-15 (Cu-disodium-EDTA) derived from ethylenediaminetetraacetic acid), containing 148 grams of copper per product. The first treatment of potassium phosphate was given to the plants when the plants had reached 33 days from sowing date and the last treatment was at 218 days from sowing date. Interval of treatment was 5 days. Copper was given at the first time when the plants age had reached 36 days and the last treatment was at the plants age of 204 days with an interval of 21 days. They were given to plants by spraying.

2.4. Inoculum preparation and inoculation of *P. infestans*
Tomato leaves infected with *P. infestans* were picked from farmer’s land in Berastagi, Karo Regency, North Sumatera, and Indonesia. Sporangium on leaves was collected by brush and dissolved in distilled water. Inoculum was measured by haemocytometer and adjusted to a concentration of 1 x 10^4/ ml [11]. The inoculum was then cooled at temperature 4 °C for two hours to stimulate zoospore out from sporangia [12]. Inoculum was then incubated at room temperature for 30 minutes before being inoculated by spraying to plants. Inoculation of *P. infestans* was carried out at the plant age of 37 days or seven days after moving to the greenhouse. Inoculation was performed through spraying to plants at 6:00 p.m. Plants were covered with transparent plastic for 7 days and shaded with 50 % light interception screen to maintain humidity around the plants.

2.5. Experimental design
This study was set in a completely randomized design with two factors. The first factor was copper at dose 0 % and 0.07% while the second factor was potassium phosphate with four doses of 0 %, 0.4%, 0.8% and 1.2%. Each treatment consisted of seven plants with 2 sample plants.
2.6. Disease assessment
Disease severity was observed one week after tomato plants were inoculated by \textit{P. infestans}. Number of observation was two times and the interval of observation was 7 days. Disease severity was determined from the first 10 leave stalks. The disease severity scale was presented in the Table 1 [11]. Calculation of disease severity (DS) was by the formula: \( \text{DS}=\sum (n_i \times v_i)/N \times V \times 100\% \) as following:

| Table 1. Disease severity assessment scale. |
|-------------------------------------------|
| 0 | No symptom |
| 1 | 01 to 10\% leaf area affected |
| 2 | 11 to 20\% leaf area affected |
| 3 | 21 to 40\% leaf area affected |
| 4 | 41 to 70\% leaf area affected |
| 5 | 71 to 90\% leaf area affected |
| 6 | 91 to 100\% leaf area affected |

2.7. The plant dry weight
A sample was taken from destructive sample plants consisted of leaves, stems and roots on a 63-day-old plants at the beginning of generative stage. The samples were dried at a temperature of 70-80\ºC for 3 days until its reached constant weight.

2.8. The plant height
The plant height was measured three times starting at the plant age of 114 days with interval 7 days.

2.9. The plant stem diameter
A stem sample was collected in 217 days-old plants after 9th times treated by Cu. The samples were taken in the middle position of the main stem, which was at the 30th leaf stalk. The staining method used was as described by [13]. The next step was to heat the prepares with Bunsen fire, continued with adding one to two drops of floroglucinol 2\% solution, incubated for about 10 minutes, air dried and put a few drops of HCl into the prepares. The stem tissue of the propagate would then turned to violet colour if it was containing lignin.

2.10. Data analysis
Data were analysed using ANOVA’s procedure by excel. Mean comparisons were conducted using Duncan’s Multiple Range Test at 0.05 probability level.

3. Results and Discussion

3.1. Disease severity
Disease severity of \textit{P. infesting} on the tomato plant was significantly decreased by the application of copper one week after inoculated by \textit{P. infesting but} it was not significantly reducing disease severity a week later (data not showed). Potassium phosphate effectively reduced the disease severity. Application of potassium phosphate caused significantly reduction on the disease severity of \textit{P. infesting} by 31.62 \% to 36.40\%, 40.26 to 63.65 \% at dose 0.4 \% and 0.8 \% respectively. Disease severity at control was 45.79\% to 79.01 \% (Figure 1 and Table 2).
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Figure 1. Disease severity (DS) on tomato plants treated with potassium phosphate and copper 2 weeks after infected by *P. infestans*. Different letters in the graph lines indicated significant different between dose (p≤0.05) according to Duncan’s test.

![Graph showing disease severity](image-url)

Table 2. Disease severity one week after inoculated by *P. infestans* at the plant age of 44 days.

| Potassium phosphite (%) | Cu (%) | 0.07 | Average |
|------------------------|--------|------|---------|
|                        | Control | Disease severity (%) |           |
| Control                | 58.79  | 32.80 | 45.79 a |
| 0.4                   | 17.38  | 10.95 | 14.17 b |
| 0.8                   | 5.92   | 5.13  | 5.53 c  |
| 1.2                   | 6.06   | 5.64  | 5.85 c  |
| Average               | 22.04 a| 13.63 b|         |

Cu = copper, different letters in the table indicated significant different between dose (p≤0.05) according to Duncan’s test.

3.2. The plant dry weight

Treatment of copper and potassium phosphate on tomato plants significantly affected the plant dry weight. Copper increased the plant dry weight by 6.39 gram while potassium phosphate increased the plant dry weight by 23.85, 26.05 and 32.05 gram at dose 0.4%, 0.8%, 1.2 % respectively (Table 3).

Table 3. Plant dry weight after two times treated by Cu and six times treated by potassium phosphate.

| Potassium phosphite (%) | Cu (%) | 0.07 | Average |
|------------------------|--------|------|---------|
|                        | Control | (g)  |         |
| Control                | 5.93   | 10.50| 8.22 a  |
| 0.4                   | 28.33  | 35.80| 32.07 b |
| 0.8                   | 33.43  | 35.10| 34.27 b |
| 1.2                   | 34.33  | 46.20| 40.27 c |
| Average               | 25.51 b| 31.90 a|        |

Different letters in the table indicated significant different between dose (p≤0.05) according to Duncan’s test.
3.3. The plant height
Observation of the plant height as seen in the Figure 2 showed potassium phosphite significantly increasing plant height by 132.34 cm to 147.57 cm, 168.66 cm to 187.17 cm at dose of 0.4 %, 0.8 % respectively. Copper had a noticeable effect on increasing the plant height (Figure 3).

Figure 2. Plant height on day (1)=114 day, (2)= 121 days, (3)=128 from sowing date. Different letters in the graph lines indicated significant differences between mean (p≤0.05) according to Duncan’s test.

Figure 3. Plant height on day (1) = 114 day, (2) = 121 days, (3) = 128 from sowing date. Different letters in the graph lines indicated significant differences between mean (p≤0.05) according to Duncan’s test.

3.4. Diameter of plant stem
Figure 4 showed that tomato plants treated with copper and potassium phosphite had a larger stem diameter (Figure 4A and 4B). The diameter of stem treated with copper at dose 0.07 % and potassium phosphite at a dose of 0.4%, 0.8 % and 1.2 % was 10.47 μm, 10.65 μm, 10.24 μm respectively while treated only with copper it had 9.45 μm. Plants that were only treated by potassium phosphite at a dose of 0.4 %, 0.8 % and 1.2 % had a stem diameter of 8.70 μm, 9.03 μm and 10.53 μm respectively while the control was 7.59 μm.
Figure 4. Picture A and B showed different diameter size of plant stem taken on day 217 from sowing date. C0 = control, C1 = Cu : 0.07 %. P0 = control, P1 = potassium phosphite (pp) at dose 0.4 %, P2 = pp at dose 0.8 % and P3 = pp at dose 1.2 %.

The application of potassium phosphate to tomato plant infected by *P. infestans* caused a significant reduction in the disease severity by 31.62 % to 36.40 %, 40.26 to 63.65 % at dose 0.4 % and 0.8 % respectively. This study result was in accordance with the following studies: [14] suggested that potassium phosphate at concentration of 5 μg phosphate ml⁻¹ and 50 μg phosphite ml⁻¹ in the ribeiro's modified medium inhibited the growth of the intermediate tolerance of *Phytophthora cinnamomi* by 30%-63% and 73%-94% respectively. [15] reported that seedling of lupin (*Lupinus angustifolius* L.) plants infected by *P. cinnamomi* were given 20 ml of 99% potassium phosphite through the introduction to the pot soil. The stem lesions was significantly reduced.

The tomato plant dry weight were increased by 6.39 gram treated with copper at dose of 0.07 % and increased by 23.85 gram, 26.05 gram and 32.05 gram treated with potassium phosphite at dose of 0.4 %, 0.8 %, 1.2 % respectively. This study suggested that tomato plants which was exposed to potassium phosphite had more healthier leaves thus they would have more leaf area. This would increase photosynthesis and carbohydrate production for the growth which resulted to the increase of the plant dry weight and their height. [16] suggested that leaf area of *Prunus serotina* Ehrh plants had linier correlation to its net photosynthesis. The study of [17] had shown that peanut (*Arachis hypogaea* L) plants infected by *Cercospora personatum* (Berk&Curt) caused necrotic spots or leaf damage therefore causing a decrease in photosynthesis and resulted to a low yield. [18] reported an increase in leaf area had positive correlation with the dry weight of *Arabidopsis thaliana* leaves and roots.

Tomato plants treated with copper showed an increase in stem diameter. According to [19] copper is essential nutrient involved in plant physiology processes, namely photosynthesis, respiration, permeability of water, carbohydrates distribution, cell wall metabolism and reproduction. Cu deficiency causes leaf chlorosis and tip necrosis in new leaves. A sufficient supply of copper to plant will stimulate carbohydrate levels and vegetative growth of plant. Carbohydrates have an important contribution to plant development that was related to the growth of plant stems.

4. Conclusions
Tomato plants infected by *P. infestans* showed a positive response to the application of potassium phosphite which was characterized by the reducing of disease severity of *P. infestans*, the increasing of the plant dry weight, the height and the stem diameter of the plant, while the plant's response to copper could be seen from the increase in the plant dry weight, the height and the stem diameter of the plant.
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