Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.4, No.24, 2014

The Increase in Effectiveness of Broccoli Waste as Bio-Fumigant to Control Ralstonia Solanacearum on Tomato (Solanum lycopersicum L.)

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Abstract
The use of synthetic bactericide can cause the death of natural enemies and pathogenic resistance. Innovation is needed to replace methyl bromide solution. Research to find biofumigan compounds as an alternative to methyl bromide is considered essential. Waste broccoli biofumigan known as a source because it contains glucosinolates (GSL). If out of the plant tissue, the GSL will be hydrolyzed to compounds that are toxic to pests and pathogens. The research aimed to obtain optimum dose for broccoli waste to control the attack of *Ralstonia solanacearum* disease and increase tomato yield. Research was conducted at Screen House of Faculty of Agriculture of Islamic University of Malang on February - June 2014. The research was conducted experimentally using completely randomized block design with three replications. Research result indicated that the application of broccoli waste of 400 g per 5 kg of soil has able to extend the incubation period to 11 days, decrease disease incidence of 86.18%, decrease the population of *Ralstonia solanacearum* 75.45%, and increase plant yield of 129.29% and number of plant fruit of 13.87%.
Keywords: effectiveness, plant waste, bio-fumigant, control, *Ralstonia solanacearum*

1. Introduction

Tomato (*Solanum lycopersicum* L.) can be planted in lowland to highland. Various problems can restrain any efforts to increase production and yield quality of tomato planting. One of important obstacles is the emergence of wilt disease caused by bacteria *Ralstonia solanacearum*. Result from field observation shows that the disease has caused the loss of fresh fruit around 5-100% (Nurjajani, 2011; Hartman *et al*., 1993; Tshu *et al.,* 1993). Various control efforts have been conducted, among others, using resistant varieties (Arlat *et al*., 1993), rotational cropping and the use of bio-agents (Semangun, 1989). Farmers have accustomed to the use of synthetic bactericide to control *Ralstonia solanacearum*. Continual use of bactericide solution and in large doses can cause the death of natural enemies and pathogenic resistance. This condition is unbearable considering that Indonesia has ratified WTO agreement in which every country member should apply security system of food security from dangerous chemical residue. Therefore, other alternatives are needed to control wilt disease of bacteria *Ralstonia solanacearum* without worsening environmental pollution problems.

One of promising alternatives for control is using bio-fumigation utilizing waste from plant of *Brassicaceae* family. In addition to its role as bio-fumigant for soil contagious pathogen, pest and weeds (Rosa *et al*., 1977), plant waste is used to add organic material content in soil. Waste of broccoli functions as a bio-fumigant and does not eradicate microbe as methyl bromide or other pesticides do. When plant waste is dissolved and embedded in the soil, hydrolyze process of GSL (glucosinolate) is occured and toxic solutions are formed. Those solutions, among other is ITS (isothyocianate), are expected to function as bio-fumigant to control soil pathogen *Ralstonia solanacearum* (Artby *et al*., 2005; Kirkeegard, 2007). The organic matter decomposition will improve soil fertility and soil infiltration (McGuire, 2003), supply nitrogen for plant (Thorup *et al*., 2003), preserve soil quality and health and support soil productivity (Mag Doff and Weil, 2004) and sustain water, especially in sandy soils (Stevenson, 1994).

Although many evidences indicate that ITS (isothyocianate) is a solution having bacteriostatic effect; however, it requires fairly high concentration of ITS to achieve the level. Therefore, the optimum doses of broccoli waste need to be determined. Based on the description, a research is needed on the increase of effectiveness of broccoli waste as bio-fumigant to control *Ralstonia solanacearum* on tomato (*Solanum lycopersicum L.*).

The research aimed to (1) obtain the best doses of broccoli waste to control the attack of disease *Ralstonia solanacearum*; (2) obtain the best doses of broccoli waste to increase tomato yield.

2. Materials and Method

2.1 Research Location and Time

Pot experiment was conducted in Screen House of Agro-technology Department, Faculty of Agriculture Islamic University of Malang. The altitude of location is 460 m above sea level. Type of soil is clay with average daily temperature of 23.5 C. The research was started on February to June 2014. The observation on population density of *Ralstonia solanacearum* was conducted at Laboratory of Biology – Faculty of science Brawijaya University.

2.2 Research Method
The research was conducted experimentally using completely randomized block design with 8 treatments: A0 = positive control (without *R. solanacearum* and broccoli waste), A1 = negative control (*R. solanacearum* and broccoli waste), A2 = *R. solanacearum* + broccoli waste of 100 g, A3 = *R. solanacearum* + broccoli waste of 200 g, A4 = *R. solanacearum* + broccoli waste of 300 g, A5 = *R. solanacearum* + broccoli waste of 400 g, A6 = *R. solanacearum* + broccoli waste of 500 g, A7 = *R. solanacearum* + bactericide Agrept. Every treatment had ten sample plants and three replications.

2.3 Preparation for pathogen *R. solanacearum*
Isolate of *R. solanacearum* used was the result of isolation of tomato plant in Donowarih Village, Karangploso, Malang attacked by *R. solanacearum*. Purification isolation and propagation of *R. solanacearum* was using medium of TZC (2,3,5-*triphenyl tetrazolium chloride*). Population density for inoculation was 2.63 x 10^8 cfu.mL^{-1} measured with spectrophotometer on OD 600.

2.4 Research
2.4.1 Preparation of Plant Material
Tomato seeds from variety of Lentana F1 were planted in nursery bed with soil medium + sand + compost with ratio 1:1:1. The seeds were previously sterilized using hot vapor for three hours. After ten days, the seedlings were removed to nursery glasses with one seedling per glass.
2.4.2 Preparation of Plant Medium
Plant medium used was soil:sand:organic material of chicken manure with ratio 2:1:1. The medium was sterilized first using steam for three hours. Plant medium of 5 kg was inserted in a polybag and anorganic fertilizer of NPK (1:1:1) of 6 gram was applied per polybag.
2.4.3 The Application of Broccoli Waste
Broccoli waste was chopped with average length of 2 cm, mixed thoroughly and covered with transparent plastic for one week to avoid evaporation of volatile solutions. Tomato seedling was transplanted in the age of one month in the nursery. The average height of seedlings during transplanting was 10 cm and had four leaves.
2.4.4 The Application of Pathogen *R. solanacearum*
Pathogen *R. solanacearum* was innoculated when the plant reached one week of age after transplanting with concentration of 10^8 cfu.mL^{-1} of 20 ml. Inoculation was conducted by wounding plant roots using scapel.
Observed response in this experiment consisted of: effectiveness of bio-fumigation control, population of pathogen bacteria, and disease incidence and calculated based on Sinaga (2006) with the following formula:

KP = n/N x 100%
KP = incidence of disease (%)
n = number of wilt plant
N = number of observed plant

Components of yield observed were fruit yield and number of fruit per plant on harvest time.

2.5 Statistical Analysis
The collected data were analyzed using The Analysis of Variance (ANOVA) , where Least Significant Difference (LSD) test at level ($p < 0.05$) followed when significant influence was present. Statistical analysis Regression and correlation analysis were conducted using minitab version 16.

3. Result and Discussion
3.1 Pathosystem Component
Based on research result (Table 1), it is known that the application of broccoli waste as bio-fumigant has significant influence ($p < 0.05$) on pathosystem components: incubation period, disease incidence, population density of *Ralstonia solanacearum* and effectiveness of broccoli waste as bio-fumigant

Table 1 indicates that the application of broccoli waste gave longer incubation period compare to treatment without broccoli waste. Disease symptoms of *Ralstonia solanacearum* occured between 13-26 days after pathogen inoculation. The application of broccoli waste is able to decelerate time needed for pathogen to infect the plant since penetration until the occurrence of wilt symptoms on tomato leaves. Research result showed that the more amount of broccoli waste applied had able to decelerate the occurrence of disease to 11 days. There was a correlation between broccoli waste doses with incubation period with R^2 value of 0.924 and the optimum dose obtained to extend incubation period was 450.6 g.

Research result also found that there was a correlation pattern between doses of broccoli waste and the

level of attack symptoms in 35 days after planting. Generally, it can be described that the maximum doses of broccoli waste to decrease the disease incidence was 370 g with R^2 = 0.930. Plants applied with broccoli waste will experience a decrease in disease incidence of wilt of 59.97% - 86.18% compare to plants without broccoli waste application. It is assumed to be related to the resulted isothyocianate (ITS) solution. Yulianti (2009) reported that *Brassicaceae* family can be used to control soil contagious pathogen. Further, McGuire (2003) explained that when broccoli waste is chopped and embedded in the soil, hydrolysis process of Glucosinolat (GSL) is occurred and toxic solutions formed. These solutions are expected to function as bio-fumigant to control the attack of *Ralstonia solanacearum*. Glucosinolate hydrolysis will produce bactericidal and fungicidal isothyiocianate. In addition, Yulianti (2009) stated that high concentration of isothyocianate is needed in order to be bactericidal and fungicidal in nature. In this research, the concentration of isothyocianate was not measured; however, it is believed that 370 g of doses could produced higher isothyocianate concentration than other doses.
Observation result on final population density of pathogen *Ralstonia solanacearum* showed that tomato plants applied with broccoli waste had able to reduce the number of final population of pathogen *Ralstonia solanacearum* of 24.8% - 79.45%. It is related to the side effect of broccoli waste application. The application was able to increase the number of soil microbe resulted during decomposition process. The increase in the number of soil microbe also caused the increase in antagonist microbe that in turn will make pathogen unable to control the available space and nutrient in maximum. During decomposition process, the composition of microbe communities in soil will change into composition that is more complex; therefore, natural balance will be formed. In addition, Smolinska (2009) stated that there are certain microorganisms live in roots and produced myrosinase enzyme that help in increasing isothyocianate production.

Level of control effectiveness using broccoli waste on wilt disease of *Ralstonia solanacearum* in this research was high. The application of broccoli waste more than 200 g to 500 g was able to increase the control effectiveness of 66.6% - 125% compare to other plants applied with broccoli waste of 100 g; whereas the maximum control effectiveness was gained with dose of 428 g.

3.2 Tomato Plant Yield

The application of broccoli waste as bio-fumigant has significantly influence the weight of fruit per plant and number of fruit per plant. There was a tendency that the more the amount of broccoli waste applied the higher the weight of fruit per plant (Figure 1).

Plant with application of broccoli waste will have an increase in fruit weight per plant of 76.73% - 132.74% and in the number of fruit per plant of 57.69% - 130.32% compare to other plants without broccoli waste application. There was a correlation pattern between broccoli waste doses and tomato fruit weight resulted with R^2 = 0.97 (Figure 2). From the equation in Figure 2, the optimum dose of broccoli waste to produce maximum fruit weight was 399.5 g.

Figure 2 shows that the increase of broccoli waste up to 399.5 g has able to increase fresh weight yield per plant. It indicates that broccoli waste in that dose is not only functions as isothyocianate producer but also as the source of organic matter to improve physical, biological and chemical condition of soil (Istiyanto, 2009). Yulianti (2004) reported that when the concentration of isothyocianate in soil is very low or undetected, broccoli waste will act as nutrition supplier for decomposer microorganisms which could act as antagonist microorganism. Antagonist microorganisms are able to reduce the attack of wilt disease and increase nutrition absorption by plant. There was a correlation pattern between the level of disease incidence and the yield of tomato fruit weight with R^2 = 0.99 (Figure 3).

Analysis on C/N broccoli waste has resulted 12.1; therefore, it is considered as organic material that easily to decompose and mineralization process takes place quickly. The mineralization has caused the available nutrient can be used to support plant growth and yield. Evan and Farquhar (1991) stated that the availability of sufficient nutrient for plant growth will make the metabolism process in plant run normally that in turn will influence photosynthesis and respiration processes. Further, Getachew et al (2011) added that the availability of nutrient and organic carbon in soil with application of organic material would increase yield and reduce the attack level of *Rolstania solanacearum* wilt on tomato.

4. Conclusion

Research result shows that: the increase in broccoli waste doses from 100 to 500 g will decrease the disease incidence of *Ralstonia solanacearum* wilt on tomato plant of 59.9% - 86.18%. Optimum dose of broccoli waste was 399.5 g that able to produce maximum fruit weight per plant. The application of broccoli waste can increase the yield of fruit weight per plant of 76.73% - 132.74%. The highest effectiveness of bio-fumigant using broccoli waste was achieved with 428 g of dose. Therefore, it is suggested that the application of broccoli waste in optimum dose can reduce disease incidence of *Ralstonia solanacearum* wilt and increase tomato yield. However, further researches are needed to find out the amount of isothyocianate solution released by other *Brassicaceae* families and its response on various tomato plants.

For the development of further research needs to be done isothyosianat measured levels (ITC) released by several sources biofumigan of some family Brassicaceae. Research on the duration of time it takes the plant family Brassicaceae in ICT-releasing compounds in the soil that are toxic to pathogens is also important.

5. Acknowledgement
The authors would like to thank the Directorate General of Higher Education of the Ministry of Education and Culture for the support for Competitive Research Grant Contract No. 016/SP2H/P/kM/2014 fiscal year of 2014, Agro-technology Department of Faculty Agriculture - Islamic University of Malang, Laboratory of Biology - Faculty of Science Brawijaya University and all parties who help the research.

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Table 1. Effect of doses of broccoli waste application of the components pathosystem

Doses of broccoli waste application	Incubation period (days)	Disease incidence (%)	The effectiveness of biofumigant (%)	*R.solanacearum* population ($x10^5$)
without *R.solanacearum* and broccoli waste	16.97 b	7.10 a	-	0.71 a
R.solanacearum without broccoli waste	13.33 a	63.67 c	-	12.02 f
R.solanacearum + broccoli waste 100 g	21.27 cd	25.52 b	44.00 a	9.03 e
R.solanacearum + broccoli waste 200 g	22.60 de	15.43 ab	73.30 b	6.71 de
R.solanacearum + broccoli waste 300 g	24.07 ef	12.53 a	85.30 b	6.04 cd
R.solanacearum + broccoli waste 400 g	25.67 f	10.57 a	89.30 b	3.41 bc
R.solanacearum + broccoli waste 500 g	24.60 ef	8.80 a	92.00 b	2.47 ab
R.solanacearum + Agrept	20.13 c	12.27 a	88.00 b	1.35 ab
LSD 5%	2.44	10.68	28.44	2.66

Remarks: the numbers with the same letters of the same column indicate not significant difference according to LSD at α = 5%

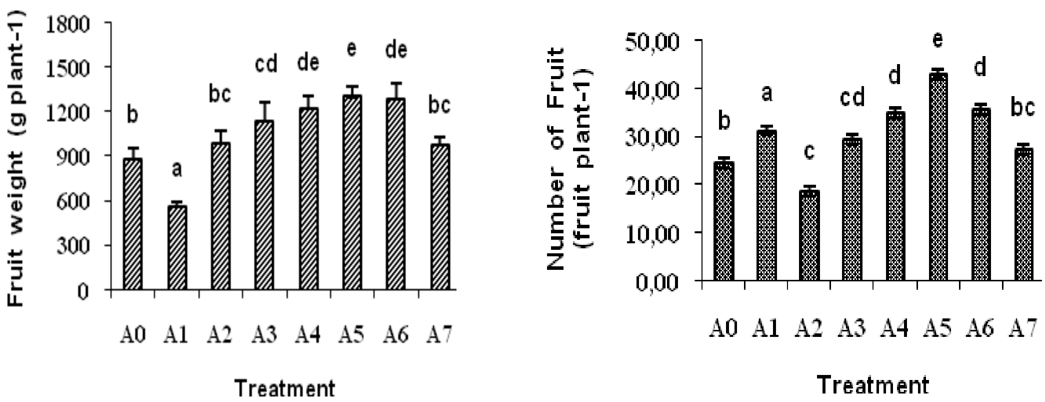

Figure 1. Fruit weight and number of fruit per plant in various treatment of waste broccoli doses

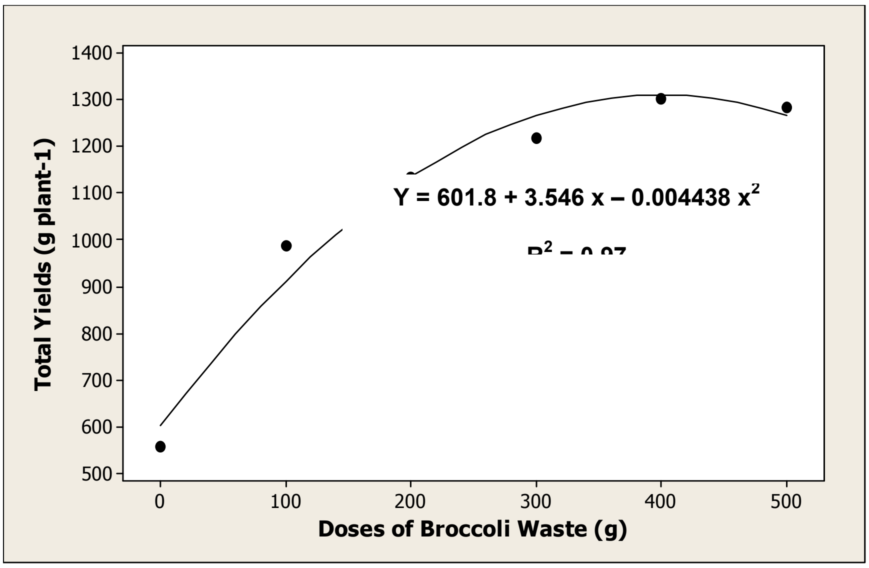

Figure 2. Correlation between doses of broccoli waste and total yields

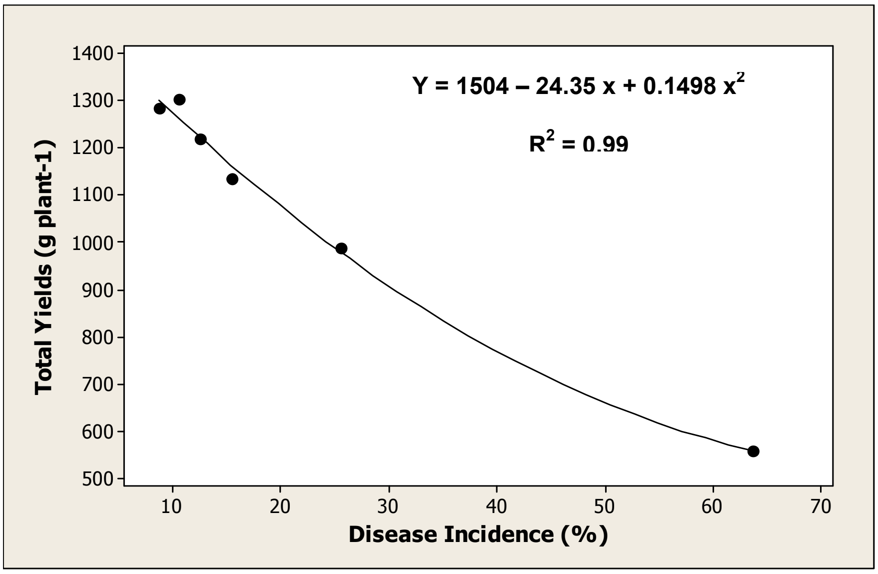

Figure 3. Correlation between disease incidence and total yields