Dual Inoculation of Azotobacter chroococcum and Trichoderma harzianum To Control Leaf Blight (Rhizoctonia solani) and Increase Yield of Choy Sum

Agusthinus Marthin Kalay, Reginawanti Hindersah, Abraham Talahaturuson, Andrias Izaac Latupapua

Leaf blight epidemic of choy sum (Brassica rapa L.) caused by Rhizoctonia solani is always happen in the intensive vegetable area of Ambon Bay during rainy season. The use of chemical pesticides for the control of leafblight diseases is not effective when diseases attack is massif. Biological control will be another way to suppress diseases incidence and will play an important role in the future agriculture. The objective of this field experiment was to determine effect of biofertilizer Azotobacter chroococcum and biological agents Trichoderma harzianum on the change of diseases intensity caused by Rhizoctonia solani, as well as yield of choy sum (Brassica rapa L.). Field experiment was conducted in Entisols, choy sum was sprayed by A. chroococcum and T. harzianum either in single or dual inoculation. The experiment showed that both single and dual inoculation of A. chroococcum and T. harzianum decreased choy sum leaves damage caused by R. solani. Controlling diseases by using of dual inoculation decreased damage intensity up to 10.77%, but statistically did not increase yield. Change in both Azotobacter and Trichoderma population in soil after harvest showed that synergistic interaction was happen between the two beneficial microbes.

Keywords: Azotobacter chroococcum, choy sum, Rhizoctonia solani, Trichoderma harzianum

1. INTRODUCTION

Plant Growth Promoting Rhizobacteria (PGPR) serves as biofertilizer, biostimulan, and bioprotector. The last function causes PGPR application is also an alternative way to partially replace chemical pesticides. Microbial biofertilizer which are widely used commercially, especially in South Asia are Azotobacter. Mechanisms of rhizobacteria Azotobacter to enhance plant growth is through nitrogen fixation and phytohormones synthesis. Recently, Azotobacter ability in suppressing the intensity of pest attack has been documented by several researchers. In the future, the use of microbial biofertilizers which also play a role in biocontrol of plant diseases and pests is important to overcome the problems caused by the use of chemical fertilizers and pesticides.

Azotobacter vinelandii, produces growth hormone and also antifungal which inhibit growth of plant diseases causing wilting Fusarium oxysporum.

[1] Azotobacter chroococcum isolated from pea rhizosphere, produce both phytohormones and antifungal that inhibit pathogen Alternaria alternata and Fusarium oxysporum.[2] In vitro experiment showed that Azotobacter's antifungal activity had inhibitory effect against Aspergillus flavus, Cercospora sp. and F. oxysporum at a high concentration of Azotobacter's culture, [3]. PGPR has been documented to induce plant resistance to disease. The data obtained showed that the number of plants with Cucumber Mosaic Virus (CMV) symptoms is smaller in plant sprayed by Azotobacter inoculant compared to that with Pseudomonas inoculant [4]. However information about the capacity of Azotobacter to control leaf blight caused by Rhizoctonia solani is limited.

Rhizoctonia solani is a fungusthat attacks the cause of the fallseedlings plant seeds in vegetable production, including choy sum. In older plants, R. solani also caused tole leaf blight disease. Biological agent that has been studied elsewhere is Trichoderma sp. which is antagonistic to R. solani.

Soil born fungi Trichoderma easily found in almost all soil type, and has an ability to control pathogenic fungus as well as patogenic nematode in agricultural soil. Several research showed that Trichoderma sp inhibit growth of...
patogenistic fungi and nematode [6],[7],[8], [9],[10]. The aim of this experiment was to determine effect of biofertilizer Azotobacter chroococcum and biological agents Trichoderma harzianum on the change of intensity damage caused by Rhizoctonia solani, as well as yield of choy sum (Brassica rapa L.) in a field experiment.

2. METHODS

2.1 Field Experiment

Experiments were carried out in the vegetable field at Wayame Village, Baguala District of Ambon City in July-August 2014 at the rainy season. Previously, in experimental field and adjacent field, green cabbage were attack by leaf blight disease resulting in significant decreased of yields. Soil in experimental field was sandy clay loam Entisols with pH of 5.8; and contained 1,67% Organic-C, 0.12% organic-N, 9.08 mg/100 g available P 2O5, 7.86 ppm total P 2O5, and 35.01 mg/100g total K2O.

Soil structure at 30 cm depth was crumb and suitable for leafy vegetable production, while at deeper than 30 cm, soil contain clay in significant amount. Soil was low in fertility, although total K2O content was medium. Lowland choy sum cv Shintagrown in this experiment could be harvested at 20-25 days after planting with shoot weight of 200-250 g and potential yield of 20-25 t/ha.

2.2 Biological Materials

Liquid biofertilizer A.chroococcumis prepared by Soil Biology Laboratory, Faculty of Agriculture, Universitas Padjadjaran. Liquid Inoculantwas made on molasses-based liquid medium to induce the production of exopolysaccharide, Liquid Inoculant was made on molasses-based liquid medium to induce the production of exopolysaccharide, with a density of 108 cfu/mL. A pure culture of Trichoderma harzianum was obtained from Plant Disease Laboratory, Faculty of Agriculture, University of Pattimura. Pure culture of T. harzianum was prepared by using composted solid waste sago, bran, husk(1:1:1; v:v:v) in 9 cm petridish. One plate of T. harzianum pure culture was mixed with 250 mL of sterilized aquadest to obtain fungal population of 106 conidium/mL.

2.3 Experimental Set up

Soil preparation was carried out by using hand tractor at about 20 cm depth before four 3mx4m trial plots were made, distance between plots was 35 cm. Hen manure at the rate of 20 t/ha was mixed with topsoil by using hand tractor. 14 day old choy sum transplants were grown in four plots at space of 20 cm x 20 cm. Individual plot was treated with A.chroococcum and T.harzianum either single or dual inoculation by using foliar application at approximately 20 mL per plant. Crops in control plot was sprayed with water without microbes. Microbial application was carried out at 5 and 15 days after planting and plants were maintained for 22 days after planting (dap).

At the end of experiment, diseases intensity due to R. solani diseases was calculated by using, Azotobacter and Trichodermapopulation was count and, and plant productivity. A total of 10 plants and soil samples taken by the method of diagonal intersection without plants in the plot.

2.4 Diseases Intensity

The intensity of disease due to R. solani attack was determined by using formula: I = Σ(n.v)/(Z.N) x 100 %, where: I= Diseases Intensity(%), n= number of affected leaves, v= diseases score, N= Number of observed leaves, Z= Value of the highest scale of the damagescore. Number of leaves were observed as follows: 0=Nodamage was observed on the leaves, 1= Extensive damage ≤25% of the leaves were observed, 2= Extensive damage 25% -50% of the leaves were observed, 3= Extensive damage 50% -75% of the leaves were observed, and 4= Extensive damage >75% of the leaves were observed [11].

2.5 Microbial Population

Determination of population was done by using Dilution Plate Method in Vermani media (10 g sucrose, 1.0 g KH2PO4, 1.0 g MgSO4.7H2O, 0.5 g NaCl, 0.1 g of CaCO3; 0.1 g NaNO3; 0.1 g FeSO4; 10 mg Na2MoO4; 1 L aquadest according to [12]. One gram of soil samples was diluted with 9 mL of distilled water and serial dilutions were made up to 105. Into sterile petri dish, 0.2 mL of suspension from 103 diluted culture was added and 20 mL of media Vermani was poured and mixed with soil suspension. After homogenized manually, the culture was incubated at 30 °C for 2-3 days.

Trichodermapopulationmeasurements was performed by the same method of Azotobacter, using Trichoderma specific media containing 200 mL of potato extract, 0.2 g of CaCO3, 0.2 g MgSO4 4.20g of dextrose, 1 L of distilled water.

2.6 Statistical Analysis

Analysis of variance was used for statistical analysis. The significance level of P = 0.05 was used throughout, using SigmaStat computer software [13].

3. RESULTS AND DISCUSSION
Choy sum in Maluku was harvested at 14-22 days after planting (dap) since Ambon community prefer to consume smaller size of choy sum with soft texture. The choice of location is based on naturally high incidence of R. solani attack on choy sum before experiment. Field experiments conducted at the end of the rainy season, the symptoms of leaf blight was showed by 10%-15% population at the first week after planting (Figure 1); so that contact pesticides are given in one and two weeks after planting to avoid plant growth failure.

![Fig. 1. Leaf blight symptoms on choy sum](image)

Based on F test, diseases intensity on the control plot was higher than plots with biological agents. Inoculation of T. harzianum without and with A. chroococcum were able to reduce the diseases intensity caused by R. solani up to 34.6% and 40.7% respectively (Table 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Diseases intensity (%)</th>
<th>Plant height (cm)</th>
<th>Shoot weight (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.8a</td>
<td>29.8</td>
<td>26.2</td>
</tr>
<tr>
<td>A. chroococcum</td>
<td>17.9ab</td>
<td>30.4</td>
<td>28.3</td>
</tr>
<tr>
<td>T. harzianum</td>
<td>14.9b</td>
<td>32.7</td>
<td>34.1</td>
</tr>
<tr>
<td>A. chroococcum and T. harzianum</td>
<td>13.5b</td>
<td>31.7</td>
<td>33.2</td>
</tr>
</tbody>
</table>

Mean values with different superscript letters in the same column differ significantly (p < 0.05).

Inorganic fertilizers were not given to any research plots. At harvest time, average fresh shoot weight was much lower than that of choy sum in farmer’s field since they gave recommended NPK fertilizer. Plant height was not differ from the average height choy sum in farmer’s field, 39.9 cm. Entisols fertility in the study area was low; contain low total N as well as available P, but moderate in total K. To avoid low yield, in the future the addition of level doses of inorganic fertilizers might be useful to provide macronutrient before biological agent and biofertilizer take a part in plant nutrition.

Positive effect of Azotobacter on plant growth and yield has been described. Azotobacter density in soil positively correlate with total N. Azotobacter grow in rhizosphere improve plant growth by exerting beneficial effects through nitrogen (N₂) fixation [14] and phytohormone synthesis [15]. The significant role of Trichoderma as biological control of plant diseases have been reported. Trichoderma produce volatile and nonvolatile antibiotics which were antagonistic with other microbes [15]. Other Trichoderma antagonistic mechanism is the production of hydrolytic enzymes and proteases that control the activity of R. solani[17]. Table 2 showed that dual inoculation of T. harzianum and A. chroococcum control diseases intensity might be due to capacity of Azotobacter to control fungi through the production of anti-fungal [2][3] and induction of plant resistance to disease [4]. In this experiment, dual inoculation might induce mutual interaction between both microbe and hence caused a decrease in crop damage by R. solani.

By using a composite sample, we measured population of biological agents soil (Table 2). There was not typical pattern of change in both population following inoculation. Effect of A. chroococcum and T. harzianum inoculation either single or dual inoculation increased Trichoderma population and vice
versa. However Dual inoculation changed both population. Change in plant metabolism following application of A. chroococcum and T. harzianum might be a cause of change in composition of microbe in rhizosphere.

TABLE 2

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Azotobacter (x 10⁴ cfu/g)</th>
<th>Trichoderma (x 10⁴ cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0,5</td>
<td>0,5</td>
</tr>
<tr>
<td>A. chroococcum</td>
<td>1,5</td>
<td>9,0</td>
</tr>
<tr>
<td>T. harzianum</td>
<td>32,5</td>
<td>1,00</td>
</tr>
<tr>
<td>A. chroococcum and T. harzianum</td>
<td>17,5</td>
<td>13,00</td>
</tr>
</tbody>
</table>

In this field experiment, Azotobacter and Trichoderma clearly reduced diseases intensity. In addition to having direct activity against phytopathogen, several Trichoderma produce components that alter the metabolism of the host; increase crop productivity, lateral root growth through the mechanism of auxin and produce substances which are analogous to the indole acetic acid [18]. Trichoderma harzianum improve the regulatory proteins that play a role in carbohydrate metabolism and photosynthesis as well as the induction of resistance [19]. In sustainable agriculture, it suggest that dual inoculation of Azotobacter and Trichoderma was a promising way to reduce the used of chemical pesticide and subsequently maintain soil environment health.

4. Conclusion

Azotobacter chroococcum and Trichoderma harzianum either in single or dual inoculation significantly reduced diseases intensity but did not affect plant height and fresh weight of individual shoot of choy sum.

References


