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

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Leaf blightepidemic 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 supress 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 diseasesintensity 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 dammage causes by *R. solani*. Controlling diseases by using of dual inoculation decreasd 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 microbe.

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

1. Introduction

PlantGrowth PromotingRhizobacteria(PGPR) serves asbiofertilizer, biostimulan, andbioprotector. The last functioncauses PGPRapplication is also an alternativeway to partially replacechemical pesticides. Microbialbiofertilizer whicharewidely usedcommercially, especially inSouthAsiaare*Azotobacter*. Mechanisms of rhizobacteria*Azotobacter*to enhance plant growth isthrough nitrogen fixation and phytohormones synthesis. Recently, *Azotobacter*ability in suppressingthe intensity ofpest attackhas beendocumentedbyseveralresearchers. In the future, the use ofmicrobialbiofertilizerswhich alsoplay a rolein the biocontrol ofplantdiseasesandpestsis important toresolve theproblemscaused bythe use ofchemical fertilizers and pesticides.

Azotobacter vinelandii, produces growth hormone and also antifungal which inhibit growth of plant disesase causing wilting *Fusarium oxysporum*:

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[1]. *Azotobacter chroococcum* isolated from pea rhizosphere, produce both phytohormones and antifungal that inhibits pathogens *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.

*Rhizoctoniasolani*is a fungusthatattacks thecause of thefallseedlingplant seeds in vegetable production, inluding choy sum. Inolder plants, *R.solani*alsoled toleaf blight disease. Biologicalagentthathas been studied elsewheretocontrolling*R. solani*isantagonistic fungal*Trichoderma*.

Soil born fungi *Trichoderma*is easily found in almost all sop type, and has an ability to control patogenistic fungus as well as patogensitic nematode in agricultural soil [5]. Several resarch 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. METHODES

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 Entisolswith pH of 5.8; and contained 1,67% Organic-C, 0.12% organic-N, 9.08 mg/100 g avilable P_2O_5, 7.86 ppm total P_2O_5, and 35.01 mg/100g total K_2O.

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 K_2O content was medium. Lowland choy sum cv Shintagrown in this experiment could be harvested at 20-25 days after planting with shoot weight of200-250 g and potential yield of 20-25 t/ha.

2.2 Biologicalmaterials

Liquidbiofertilizer*A.chroococcum*isprepared bySoil Biology Laboratory,Faculty of Agriculture, Universitas Padjadjaran. Liquid Inoculantwas madeonmolasses-based liquidmediumtoinducethe production ofexopolysaccharide, with a density of10^8cfu/mL.*Azotobacter* inoculant was diluted to 0,5% before applied so that the cell density in inoculant reached up to 10^8 cfu/mL.

*Trichodermaharzianum*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 10^8 conidium/mL.

2.3 Experimental Set up

Soil preparation was carried out by usinghandtractorat about20cm depth before four 3mx4mtrialplotswere made, distance between plots was 35cm. Hen manureat the rate of20t/ha was mixed with topsoil by using hand tractor. 14 day old choy sum transplants were grown in four plots at space of20 cm x 20 cm. Individual plot was treated with *A.chroococcum*and *T.harzianum*eithersingleor dual inoculation by using foliar application at approximately 20 mL per plant. Crops in control plotwas sprayed with water withoutmicrobes. 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 intensitydue to *R. solani* diseaseswas calculated by using, *Azotobacter*and*Trichoderma*populationswas count and , and plant productivity.A total of10plantsandsoilsamplestakenby the method ofdiagonalintersectionwithout plantsintheplot

2.4 Diseases Intensity

The intensity of disease due to *R.solani* attackwas determinedby using aformula: $I = \Sigma(n.v)/(Z.N) \times 100$ %, where: I=Diseases Intensity(%), n= number ofaffected leavesineachdamage score, v=diseases score , N= Number ofobserved leaves, Z= Value ofthe highestscaleofthe damagescore. Number ofleaveswereobserved asfollows: 0=Nodamagewas observedon theleaves, 1=Extensivedamage≤25% of theleaveswereobserved, 2=Extensivedamage25% -50% of theleaveswereobserved, 3=Extensivedamageto50% -75% of theleaveswereobserved, and4=Extensivedamage>75% of theleaveswere observed [11].

2.5 Microbial Population

Determination ofpopulationwas doneby using Dilution Plate MethodinVermanimedia(10 gsucrose, 1.0gKH_2PO_4, 1.0g$MgSO_4.7H_2O$, 0.5gNaCl,0.1g of$CaCO_3$; 0.1g$NaNO_3$; 0.1gFeSO4; 10mgNa_2MoO_4; 1Lakuadestaccording to [12]. One gram ofsoilsamplewas dilutedwith0.9mL ofsterilized distilled waterandaserialdilutions was made upto10^{-3}. Intoa sterile petri dish, 0.2 mLof suspensionfrom 10^{-3}diluted culture was added and 20mL ofmediaVermani was poured and mixed with soil suspension. Afterhomogenizedmanually, the culture was incubatedat30 °Cfor 2-3days.

*Trichoderma*populationmeasurementswas performedby the same methodof *Azotobacter*, using*Trichoderma*specificmediacontaining200mL ofpotato extract, 0.2g ofCaCO3, 0.2gMgSO 4.20g ofdextrose, 1Ldistilled water.

2.6 Statistical Analysis

Analysis ofvariance 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 inMalukuwas harvested at14-22 daysafterplanting (dap)since Ambon community prefer to consume smallersize of choysum with softtexture. The choice of locationis based onnaturally highincidence of*R.solani*attackonchoy sum before experiment. Field experimentsconductedatthe end ofthe rainyseason, the symptoms ofleaf blight was showd by 10% -15% population atthe first week after planting (Figure 1); so thatcontact pesticidesare giveninoneandtwoweeksafter plantingtoavoidplant growth failure.

Fig. 1. Leaf blight symptoms on choy sum

Based on F test, diseases intensity onthe control plotwas higher thanplotswithbiologicalagents. Inoculation of*T.harzianum*withoutandwith*A.chroococcum*were enable toreduce the diseases intensity caused by*R.solani*up to34.6% and40.7% respectively (Table I). *Azotobacter*biofertilizersbiologically interacteed withTrichodermatosuppressleafblight . Both microbialtreatmentdid not affectplant heightandshootweightat harvest time. There ispotential forbothmicrobestoimproveresults, especiallythe doubleinoculationof biological fertilizersandbiological controlbutnot statistically significant. However, a decrease incropdamagemaynotoccurifthe plantsare notsprayed withchemical pesticides. This illustrates that when leaf blight symptoms was not massif, then biologicalagentscould controlthe diseasesInsevereattacks, it is necessary to ensureeffectivenessof biological agent throughresearchwithoutpesticides.

TABLEI
EFFECT OFINOCULATIONOF *A.CHROOCOCCUM*ANDT.*HARZIANUM*AGAINSTDAMAGE CAUSED BY*R.SOLANI*INTENSITY, ANDYIELDOF CHOY SUM

Treatmen	Response		
	Diseases intensity(%)	Plantheight(cm)	Shootweights(g/plant)
Control	22.8[a]	29,8	26.2
A.chroococcum	17.9[ab]	30,4	28.3
T. harzianum	14.9[b]	32,7	34.1
*A. chroococcum*and*T. harzianum*	13.5[b]	31,7	33.2

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

Inorganic fertilizers was 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 recomended NPK fertilizer. Plant height was not differ from the average height choysum 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 aviod 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 discribed. *Azotobacter*density in soil positively correlate with total N. *Azotobacter* grow in rhizosphere improve plant growth by exerting beneficial effects through nitrogen (N_2) 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 disesases 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 measurd population of biological agents soil (Table 2). There was not typical pattern of change in botth 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 chante in composition of microbe in rhizosphere.

TABLE2
POPULATIONAZOTOBATERANDTRICHODERMAINTHE SOILAFTER ASINGLE OR DOUBLEINOCULATIONOF *A.CHROOCOCCUM* AND*T.HARZIANUM*INPLANTINGGREENS

Treatmen	Population mikrobial	
	Azotobacter (x 10^4 cfu/g)	*Trichoderma* (x 10^4 cfu/g)
Control	0,5	0,5
A.chroococcum	1,5	9,0
T. harzianum	32,5	1.00
*A. chroococcum*and*T. harzianum*	17,5	13,00

In this field expriment, *Azotobacter* and *Trichoderma* clearly reduced diseases intensity. In addition to having direct activity against phytopatogen, 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 chroocccum 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.

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