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RESEARCH ARTICLE

GROWTH OF CHOY SUM FOLLOWING *AZOTOBACTER*AND *TRICHODERMA* INOCULATION

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ABSTRACT

The way to reduce agrochemicals to ensure safety food production is plant inoculation with biological agents. Bioprotectant*Trichoderma*and biofertilizer*Azotobacter* arewidely used in sustainable agriculture. The objective of this pot experiment was to evaluate the effect of dual inoculation, *Azotobacter* sp. and *Trichoderma harzianum*on the growth of choy sum (*Brassica rapa* L. var. *Parachinensis*) and available N in soil.Pot experiment was set up in randomized block design with nine combination of inoculation times and doses of carrier-based mix inoculum. All plants were maintained in green house for a month. The effect of biological agents inoculation at any application time and doses on shoot height, fresh weight and dry weight was not significant, but irrespective to statistical analysis, certain treatment increased plant growth. However, the soil treated with lower dose of biological agent at one week before transplanting significantlyincreased more N-NH_4^+ and N-NO_3^- content in soil. This pot experiment concluded that dualinoculation of *T.harzianum* and *Azotobacter* sp. bring benefits to availability of nitrogen in soil and could enhance plant growth.

INTRODUCTION

Choy sum (*Brassica rapa* L.var. *Parachinensis*) belongs to the Brassicaceae family, a kind of leafy vegetable which is consumed regularly in Asian inhabitants' dietary. In general, excessive inorganic and organic fertilizer had been used by the farmers to maintain or increase yield of choy sumin agricultural area of West Java. Fertilizer has become essential plant nutrient source to intensify the agricultural activity thus feed the growing population of society. However excessive use of inorganic fertilizers has hardened the soil due to soil organic matter degradation and further decreased the soil health. Biofertilization has been used to replace certain amount –not all- chemical fertilizers in tropical sustainable agriculture. The majority of choy sum production is carried out in low to middle altitude in West Java where the land is dominated by moderate to low fertility soil cause by heavy weathered. Adding inorganic fertilizer to such soil is necessary but the doses are should be lowered by using biocontrol, biofertilizers and organic matters. Certain microbes living in plant rhizosphere are able to stimulate the plant growth which relate to biofertilizer; and protect the plant against soil borne diseases which known as biostimulator.

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The well-known rhizobacteria that belongs to genus *Azotobacter* stimulate the plant growth through either nitrogen fixation and phytohormone. Recent studies showed that aerobic non-spore forming *Azotobacter* produce exopolysaccharide which has a significant role in soil aggregation and further nutrient uptake (Gauri *et al*., 2012). Azotobacter is easily isolated from the rhizosphere of agronomic important commodities such as maize, rice, sugarcane, vegetable and plantation crops (Arun, 2007). The biofertilizer *Azotobacter*had clearlyincrease biomass and protein content of maize (Esmaeili *et al.,* 2016). Studies report that single inoculation of *Azotobacter* generally increases the plant growth and yield since this nonsymbiotic heterotrophbacteria is able to fix dinitrogen in average of 20 *kg* N/*ha*/year (Kizilkaya, 2009). Both of liquid and solidcarrier based of inoculant *Azotobacter* increased the plant height, number of leaves, shoot length, root length and number of roots of 20 day old *Amaranthusretroflexus*; along with their chlorophyll and carotenoids content (Maheswari and Kalaiyarasi, 2015). Some of the *Azotobacter* are reported to have antagonistic potential against several plant pathogens. In the rainy season, choy sum production usually decrease due to low light intensity and poor drainage which induce the soil born disease proliferation. Certain species of Nitrogen-fixing bacteria *Azotobacter* has an antagonistic effect on soil borne pathogen. *Azotobactervinel and ii* produced antifungal substances to inhibit proliferation of*Fusarium oxysporum* which cause wilt disease (Boshale *et al*., 2013). *Azotobacterchroococcum* that isolated from rhizosphere of legume produced phytohorm one and antifungal which inhibit the growth of pathogenic fungi *Alternariaalternata* and *Fusarium oxysporum* (Mali and Bodhankar, 2009). Enhancing vegetable productivity and its quality commonly is carried out by using long term excessive use of chemical fertilizers, in certain case producing environmental pollution. The use of biofertilizers and biocontrol is a sustainable way to decrese environmental damage. Soil fungi *Trichoderma* spp. can induce plant growth by suppressing plant diseases (Van Wees *et al*., 2008). Some strains of *Trichoderma* enable to reduce the plant diseases incidence by inhibiting soil born plant pathogens, through antagonistic and mycoparasitic mechanisms (Viterbo and Horwitz, 2010). Some Trichoderma in rhizosphere showed a direct effects on plants growth, increasing their growth potential and fertilizer use efficiency (Shoresh *et al*., 2010). The objective of this pot experiment was to evaluate the effect of dual inoculation, *Azotobacter* sp. and *Trichoderma harzianum* on the growth of choy sum (*Brassica rapa* L. var. *Parachinensis*) and available N in soil.

MATERIALS AND METHODS

Pot experiment was carried out in green house inthe middle of the year 2016 at Faculty of Agriculture Universitas Padjadjaran. Two-weeks old transplants of choy sumwere grown on Inceptisols (pH 5.87, low in organic carbon 1,58%, low in total nitrogen 0,20%, very low in $P_2O_{5potential}$ and $P_2O_{5availble}$, low in $K_2O_{potential}$) in 5 kg poly bag for a month with 20 t/ha organic matter amendment; cow manure (organic carbon 33.54%, total N 1.8%, C/N 18, pH 6.21,P_2O_5 1.2%, K_2O 3.7%) prepared by Faculty of Husbandry Universitas Padjadjaran.

Microorganisms

Strain of *Azotobacter* c2a9 were isolated a year before from rhizosp here of chili grown on Entisols Ambon Bay in Maluku Province. Liquid inoculant of *Azotobacter*was prepared in liquid waste from sago flour production. The bacteria enable to fix nitrogen in free-N liquid media where ammonium and nitrate content were 20 mg/Land 508,7mg/L respectively after 3-day culture incubation. *Trichoderma harzianum* belong to Laboratory of Phytopathology Faculty of Agriculture Universitas Pattimura Ambon; isolated from cyst nematode-infested soil in Ciwidey, West Java Province. Liquid inoculant of *Trichoderma* was prepared by using molase based media. Carrier-based *Azotobacter*sp.-*Trichodermaharzianum* mixed inoculant was formulated in sago solid waste compost.

Green House Experiment

The experiment was set up in Completely Randomized Block Design which tested ten combined treatment of carrier-based inoculant treatment and application time. The dose was 2.5, 5, and 7.5 g per pot; and the application time was1) mixed with organic matter at soil preparation one week before planting; 2) at planting time, one week after organic matter application; and 3) second application time with 20 mL liquid inoculant *Azotobacter* application 10 days after planting. Control treatment was without inoculation. All plant was maintained for four weeks in the green house and fertilized with NPK inorganic fertilizer (N:P:K; 15:15:15) at 75% of normal rate. Fertilizer was applied twice at and 15 days after transplanting on the hole near the plant. To control the insect attack on choy sumleaves, broad-spectrum insecticide Decis (a unique single-isomer pyrethroid) has been used. Research dependent variable was plant height, fresh weight and dry weight; as well as available $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$. Plant parameters were measured at four weeks after planting; dry weight of shoot was weighing after being heated at 70^{o}C for two days. Soil available nitrogen weretesting by using Morgan Wolf solution. All data was subjected to analysis of variance (5% F-test) and 5% Duncan Multiple Range Test if analysis variance was significant.

RESULTS AND DISCUSSION

Plant growth

Based on the analysis of variance, the effect of *Azotobacter-Trichoderma*doses on either shoot height and fresh shoot weight did not depend on the application time. However the plant response were determined by combination treatment of doses and application time (Fig. 1). There was no consistent effect of treatments on both parameters comparing to control plant. Irrespective of statistical analysis, half of the treatment lowered theplant height (Fig 1a) and most of treatment enhanced the fresh weight (Fig 1b).

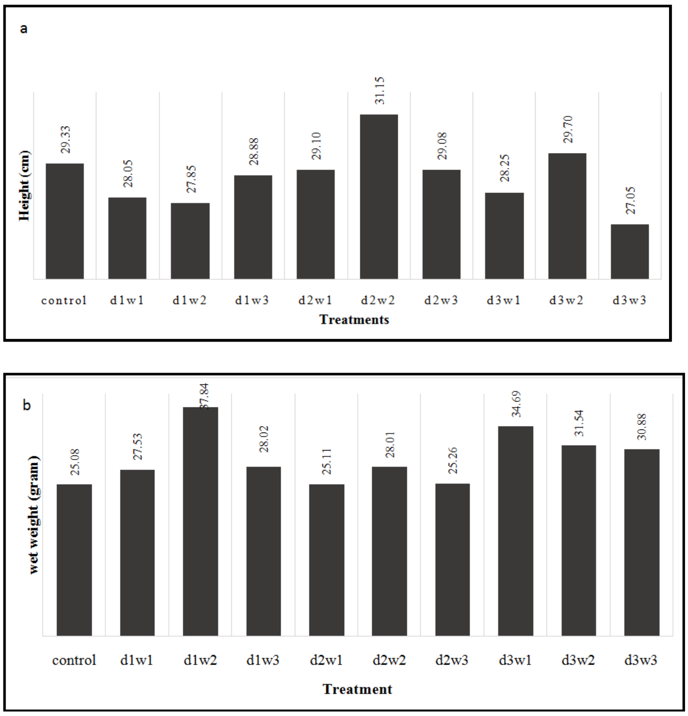


Fig. 1. The effect of *Azotobacter-Trichoderma* inoculation at different application time on height (a) and shoot fresh weight (b) of choy sumfour weeks after transplanting
d_1, d_2, d_3: biofertilizer2.5, 5.0, and 7.5 g/pot
w_1: mixed with organic matter at soil preparation one week before planting
w_2:on the hole at planting time
w_3: w_2+ 20 mL liquid inoculant *Azotobacter*per plant at 10 days after transplanting

Positive effect of biofertilizer on plant height was demonstrated when 5 gbiofertilizer has been placed on planting hole under transplant (Fig 1a), but the highest fresh weight was experienced by plant received 2,5 g biofertilizer at planting time (Fig 1b). Vegetative growthof leafy vegetable requiresloose, fertile, moist, sandy loam soils for best root growth. Many of leafy vegetables including choy sumhave shallow root systems. In this experiment, the root length was not measured, however the increasing of the shoot height and fresh weightmight be caused by the massive root growth. The importance of the root system to plant productivity has been acknowledged. *Azotobacter* are much more abundant in the rhizosphere of plants than in the bulk soil although their abundance depends on the crop species (Ponmurugan *et al*., 2012). Indole AceticAcid produced by *Azotobacter*in lag phase induced root development of *Sesbaniaaculeata* and *Vignaradiata* (Ahmad *et al*., 2005) thus *Trichoderma* defends the plant against the soil borne pathogen (Spadaro *et al*., 2005). Higher colonization of *Trichoderma* in the rhizosphere is also reported elsewhere (Ahmad and Baker, 1987; Mantha*et al*., 2013). The enhancement of plant height and fresh weight of choy sum (Fig 1) might be caused by nitrogen availability from *Azotobacter* dinitrogen fixation, and enhance the seedling growth by *T. harzianum* (Mantja *et al*, 2013).

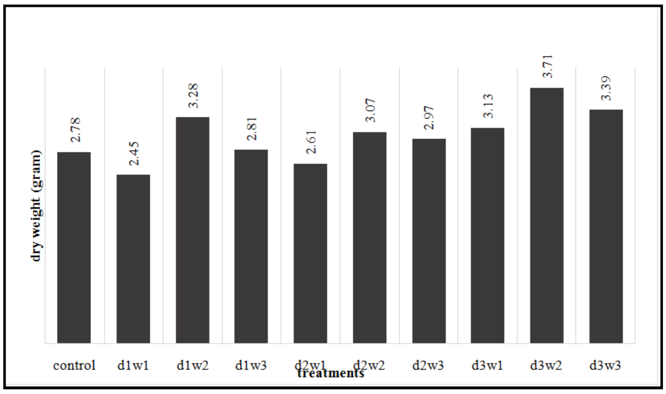
growth. The soil has low nitrogen content although the soil acidity was appropriate for choy sum growth. High content of clay create physical barrier to vegetable root growth. In this experiment manure amendment of 20 t/ha was not enough to create the best planting media suitable for choy sum growth. Plants have a high water content which depend on the amount of available water in soil. It is not easy to equalize so using dry weight as a measure of plant growth tends to be more reliable to measure photosynthesis product (Shipley and Vu, 2001).

The biomass or dry weight is important to asses the acummulation of the photosynthesis metabolyte. In this experiment, the biomass of control plant was 2.78 grams. Ignoring statistical analysis, dry weight of plant treated by most of biofertilizer application has been increased (Fig 3). Dry weight was increased due to to *Azotobacter*and *Trichoderma* inoculation. The positive effect of both biological agent on plant growth was caused by both of biofertilizationand bioprotection mechanisms. Role of *Azotobacter* as Plant Growth Promoting Rhizobacteria is recognized. *Azotobacter* also reported as an agent to control soil borne fungi.

Fig. 2. Choy sum performance at harvest time, four weeks after transplanting

The soil in this experiment has low fertility with low nitrogen content allows the proliferation of free nitrogen fixing bacteria; high nitrogen in soil do not inhibit *Azotobacter* proliferation but decrease the nitrogen fixation due to nitrogenasein activation (Kizilkaya, 2009). The part of the choy sumplant which is purchased is the shoot thus fresh weight of the shoot is important indicator. The fresh weight of the control plant was only 25,08 g and the plant treated with 2.5 g of biofertilizer on planting hole was 37.84 g (Fig 2); lower than the maximum fresh weight 150 g/plants. Leafy vegetables of Brassicaceae family grow best in soils with a pH of 5.5 to 6.5 and nitrogen is needed to support the vegetative

Antifungal substance produced by *A. vinelandii* to inhibit the proliferation of *Fusarium oxysporum* which cause wilt disease (Boshale*et al*., 2013), whereas *A. chroococcum* antifungal substance inhibit the growth of *Fusarium oxysporum* in legumes (Mali and Bodhankar, 2009). *Trichoderma harzianum*is prominent candidate of biocontrol agent against soil borne diseases since they producedcell wall degrading enzymes (Srivastava *et al*., 2015); it made the fungi be able to degrade pathogen cell. Highest dry matter was belongs tochoy sum that received higher rate of biofertilizer on planting hole. Consistent decreased of dry content was showed by plant inoculated with 20 ml of *Azotobacter* liquid inoculant at 10 days after transplanting; overuse of biofertilizer didnot give a better yield. The negative effect of *Azotobacter* on plant biomass is not yet reported. *Azotobacter* in leaf might be compete with normal leaves microflora and microbial activity which was might be correlated with loss plant biomass (decreased dry weight).



Effect of Azoto-Tricho biofertilizer inoculation at different application time on height (a) and shoot fresh weight (b) of choy sumfour weeks after transplanting d_1, d_2, d_3: biofertilizer2.5, 5.0, and 7.5 g/pot w_1: mixed with organic matter at soil preparation one week before planting w_2:on the hole at planting time w_3: w_2+ 20 mL liquid inoculant *Azotobacter* per plant at 10 days after transplanting

Table 1. The effect of *Azotobacter-Trichoderma* inoculant application with the different application time on N-NH_4^+ and N-NO_3^-in soil

Treatments	N-NH_4^+(%)	N-NO_3^- (%)
control	0.02 a	0.04 ab
d_1w_1	0.04 c	0.12 c
d_1w_2	0.03 b	0.06 ab
d_1w_3	0.02 a	0.07 b
d_2w_1	0.03 b	0.04 ab
d_2w_2	0.02 a	0.06 ab
d_2w_3	0.03 a	0.03 a
d_3w_1	0.03 a	0.03 ab
d_3w_2	0.04 c	0.04 ab
d_3w_3	0.03 b	0.02 a

Note: Numbers with the same letters were no significant based on Duncan's Multiple Range Test 0.05.
d_1, d_2, d_3: biofertilizer2.5, 5.0, and 7.5 g/ pot
w_1: mixed with organic matter at soil preparation one week before planting
w_2:on the hole at planting time
w_3: w_2+ 20 mL liquid inoculant *Azotobacter* per plant at 10 days after transplanting

Soil Nitrogen

Azotobacter has important role in nitrogen fixation to serve the nitrogen to soil in the form of ammonium and nitrate that can be uptake by the roots. The effect of biofertilizer dosage to availabe NH_4^+in soil was determined by the time of application;certain treatment increased N-$NH_4^+$and/or N-NO_3^- content in soil (Table 1). Nitrate in soil is easily leached due to the negative charge but it is mobile in soil thus immediately available for plant uptake. In soil without biofertilization, nitrate content was 0.04%. Certain treatment increased the nitratecontent; the highest one was in soil received 2.5 g mixed biological agent a week before planting. The evidence of ammonium level enhancement in soil following some biological agent treatment showed that *Azotobacter*c2a9 can fix di nitrogen in low nitrogen soil. *Azotobacter*was an indicator of the amount of soil nutrients. A research conducted in Ukraine has showed thereduced number of free-living nitrogen-fixing bacteria under the influence of nitrogen fertilizers (Mikajlo *et al*., 2014). Our research used lower rate of inorganic fertilizer which contain nitrogen, phosphorus and potassium. *Azotobacter* activity to fix nitrogen in this environment has not suppressed; although nitrogenase activity was reduced by excessive nitrogen in soil. Nitrogen from inorganic fertilizer in consumed by bacteria to proliferate their cell before they fix nitrogen when the nitrogen level in soil has reduced. Nitrogen-fixing *A. chroococcum*isolated from moderately intensified fields was higher than that from intensified field (Narayan and Kehri, 2012) which suggest that reduced inorganic fertilizer in this green house experiment was one of important factor to maintain nitrogen fixation to produce N-NH_4^+ and N-NO_3^-.

Conclusion

The effect of some combination of inoculant application time and dose on shoot height, fresh weight and dry weight was not significant, but irrespective to statistical analysis, certain treatment increase plant growth. The results showed that the optimal inoculation dose to increase N-NH_4^+ and N-NO_3^- in soil was depend on inoculation time. However, soil treated with lower dose of biological agent at one week before transplanting significantly increased more N-NH_4^+ and N-NO_3^- content in soil. This pot experiment suggested that dualinoculation of *T.harzianum* and *Azotobacter* sp. bring benefits to plant growth and availability of nitrogen in soil.

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