Original Research Article

The Competence of *Streptomyces narbonensis* and *Trichoderma harzianum* mixed as PGPM and Decomposer on Different Types of Soils

ABSTRACT

Based on the first research *Streptomyces narbonensis* and *Trichoderma harzianum* mixed was a Biological Agents (BCAs) of tomato fusarium wilting. Another research found *Streptomyces* sp. and *Trichoderma* sp can be decomposer and growth hormone producers. The purpose of *S. narbonensis* sp. and *T. harzianum* used to observe microbial competence as decomposers, PGPM of horticultural crops on different soil. Microorganism suspension was applied with a combination of *S. narbonensis* sp. and *T. harzianum* with different compositions (1:1; 2:1; 3:1 and 4:1) in liquid Glucose Potato Extract media, Microorganisms mixed was applied into three soil type Vertisol, Regosol, and Andisol. Each treatment was repeated three times. Growth of vegetative plant, the number of microorganisms and Physical chemical soil analysis, was carried out 30 days after planting. The results of the analysis soil conditions after the addition of the microorganism almost did not affect nutrient levels in the soil but only affected the available P regosol soil, increased microbial abundance that affects the decomposition of C-organic soils.

Keywords: growth hormone, soil fertility, microorganism, vegetative plant

1. INTRODUCTION

Microorganisms are critical to the sustainability of agriculture. Numerous studies have demonstrated that microorganisms used as biofertilizers, biopesticides, bioherbicides, and bioremediation all contribute to soil fertility and plant development, hence maintaining agricultural soil ecosystems in perpetuity. Biopesticides are biological insecticides that are derived from living microbes or natural ingredients. They have demonstrated pest management capabilities and are being applied globally as a result of new legislation and the evolution of resistance in pest populations [1]. The use of PGPB in conjunction with plants cultivated on marginal soil will constitute a green technology [2]. The inherent potential of PGPMs as biological agents against plant diseases has been shown [3].

Streptomyces narbonensis metabolites including antifungal, chitinases, and glucanases are versatile as a cornerstone biocontrol of fungal and bacterial soil diseases [4]. In addition to being a biological agent, it is also a microbe that degrades carbon from crop residues so that it is available to plants and decomposers of recalcitrant protein for proteolysis [5]. Previous research explains in more detail that *S. narbonensis* can oxidize ammonium to nitric acid,

also produces proteins and amino acids that effectively degrade grass straw to become a source of carbon available to plants and as decomposer converts recalcitrant protein for proteolysis [6]; [7];[8]. S. narbonensis potential to promote seedling germination, shoot, and root induced cauliflower stalk expant [9].

Trichoderma harzianum is a biopesticide and a biofertilizer that improves soil conditions and has the ability as a biological agent to pathogenic fungal with various mechanisms such as parasitism, competition, and antibiosis [10]. The functions of multi-antagonist *Trichoderma sp.* and *Gliocladium sp.* were biological agents and biological fertilizers packaged in compost as P solvent and K solvent [11]. Yaqub and Shahzad's research [12] proved that the use of *Trichoderma sp.* and *Gliocladium sp.* on sunflower seeds increased plant root growth and plant height. Combination of biological agent *S. griseorubens*, *G. virens*, and *T. harzianum* compatible inhibits *F. oxysporum* in vitro, the severity of fusarium wilt and increased fruit production [13]

Marginal soils from acid sedimentary rocks have low mineral reserves or nutrient reserves. Acidic sedimentary soils have low soil fertility. Marginal soil is characterized by textures that vary from sand to clay. Suharta [14] research results. Acidic land, Andisol and vertisol are land plots with low fertility conditions, so technological innovation is needed to improve productivity to reach a sustainable farming system. Sustainable agriculture must first focus on minimizing chemical inputs while maintaining high yields and productivity [15]. The synergies that typically result from integrating environmentally friendly techniques and products can also be used to enhance the integrated application of environmentally friendly techniques and products [16]. In this case, using beneficial microbes like *T. harzianum* and *S. narbonensis* could be a long-term solution to address these needs.

2. MATERIAL AND METHODS

2.1 Exploration and Isolation of *S. narbonensis* and *T. harzianum*.

The isolation and pathogenicity tests were conducted using a survey and descriptive methodology. Dhingra and Sinclair's techniques [17] were used as references for soil plating to isolate microbial biological agents [18]. The soil of tomatoes, protected forests Meru Betiri, and Kelud Volcano was weighed using an analytical scale to a maximum of 1 g and then diluted 10^{-4} in suspension. Additionally, 1 mL was aseptically injected and flattened onto a petri dish containing GNA (Glucose Nitrate Agar). Microbes with distinct properties and characteristics known as Actinomycetes were isolated, purified, and cultured in Petri dishes PDA (Potato Dextrose Agar). *T. harzianum* was isolated in the same method as *S. narbonensis* but at a suspension dilution of 10-7. Additionally, 1 mL of PDA was aseptically injected and flattened.

2.2 Biological Agents Preparation

Biological substances isolation using the dilution procedure: Prepare a suspension of biological agents using the dilution technique, using two blocks (each 5 mm) of *S. narbonensis* for 14 days and *T. harzianum*. For seven days, respectively, into 10 mL of sterile water in a test tube. The tube holding the microorganisms is then vortexed for two minutes at high speed. Each acquired suspension is filtered using Whatman paper. Suspension multi-antagonist was made by vortexing 6 mL *S. narbonensis* suspension and 6 mL *T. harzianum* suspension in 34 mL distillate water in the following ratios: 1:1, 2:1, 3:1, 4:1. Afterwards and calculated using the following formula:

CFU/mL= (number of colonies x dilution factor)/volume of culture plate

To quantify bacteria, tenfold serial dilutions were produced, and 100 µl of each diluent was spread on potato dextrose agar (PDA) plates. The plates were then incubated and the CFU

(colony-forming unit) was calculated [19]. Plates containing 300 or more colonies/plaques are considered TNTC (Too numerous to count). All treatments were repeated twice, and data were analyzed using SPSS software using one-way ANOVA (analysis of variance). Duncan's Multiple Range Test (DMRT) was performed to determine the value of the difference between treatment combinations with a significance threshold of p = 0.05.

2.3 Soil and Seeds Preparations

Preparing of acid soils, vertisol, and regosol soil by sterilized using steam (steamed), then the sterile soil was put into a polybag and given a multi-antagonist suspension according to treatment. Seeds of healthy plants, soaked in 50ºC warm water, then planted in sour land, vertisol and regosol in a polybag. They were then inoculated and watered every day.

2.4 Observation

The study was observed by examining the following deciding variables:
1) Tomato plant growth is measured in plant height and leaf count.
2) Efficiency of *S. narbonensis* and *T. harzianum*., used as fertilizer
3) The number of microorganisms present before and after inoculation with biological agents. Marginal soil is weighed using a 1 gram analytical scale and then diluted 10^{-4}. Additionally, 1 mL was aseptically placed and put onto Petri plates with PDA media. The number of microorganisms that are growing is then calculated.

3. RESULTS AND DISCUSSION

3.1 Tomatoes Plant Growth

The development of tomato plants in the vegetative phase was analyzed using analysis of variance for leaf and height plants. Thirty days after planting and applying a combination of BCAs *S. narbonensis* and *T. harzianum*, the average number of leaves and tomato plant height was determined. The number of leaves and height were significantly affected on regosol, Andisol, and vertisol soils. Without BCAs, the average number of leaves per plant was 4-5 lower than when *S. narbonensis* and *T. harzianum* were treated with BCAs. Six to eight strands. Additionally, the average plant height revealed that plants without BCAs were 11–12.50 cm shorter than those with BCAs for *S. narbonensis* and *T. harzianum*. 13.50 - 21 centimeters (table 1).

BCAs combination of *S. narbonensis* and *Trichoderma sp.* may serve as PGPMs, increasing the number of leaves and plant height on Andisol, Regosol, and vertisol marginal soils. This finding is consistent with previous studies indicating that *S. narbonensis* is capable of boosting the height of tomato plants through Plant Growth Promoting Bacteria (PGPB) [13]. The capacity of *T. harzianum* to increase the number of leaves and boost the development of cherry tomato plants is a result of its effect. According to Rizal et al. [20], *T. harzianum* treatment had a substantial influence on the average number of leaves on tomato plants. Numerous studies have shown a maximal increase in plant development when inoculation with PGPB as compared to uninoculated plants cultivated on marginal soils [2].

Table 1. The number of leaves and plant height on different types of soil

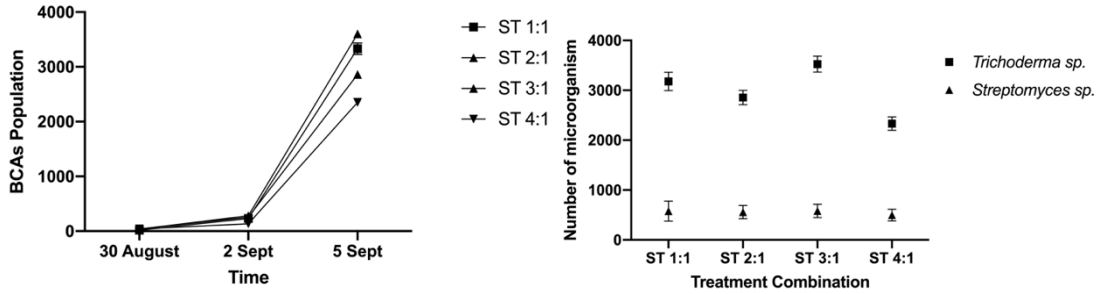
Treatment Kind of soil: Combination of BCAs	number of the leaf (sd =0,76)	height of the plant (sd =1.95) (cm)

Regosol without BCAs	4,50[a]	13,50[a]
Vertisol without BCAs	4,50[a]	15,50[a]
Andisol without BCAs	5,50[ab]	18,00[b]
Andisol 2:1	6,00[ab]	18,50[bc]
Andisol 3:1	6,50[b]	19,00[bc]
Regosol 2:1	6,50[b]	19,50[bc]
Andisol 1:1	7,00[b]	19,50[bc]
Andisol 4:1	7,00[b]	19,50[bc]
Regosol 4:1	7,00[b]	20,00[bc]
Vertisol 4:1	7,00[b]	20,00[bc]
Regosol 3:1	7,50[b]	20,50[bc]
Vertisol 1:1	7,50[b]	22,00[b]
Vertisol 2:1	7,50[b]	22,50[b]
Regosol 1:1	8,00[b]	22,50[b]
Vertisol 3:1	8,00[b]	24,00[b]

3.2 Microbial populations pre- and post-inoculation

At harvest on September 5, fungal and bacterial populations were evaluated. Fungal populations increased significantly ($p = 0.05$) in the combo treatments compared to the solo treatment. Infected *S. narbonensis* and *T. harzianum* with a combined ratio of 1:3 had the highest fungal population in the soil, followed by infected *S. narbonensis* and *T. harzianum* with a combined ratio of 1:1. On the other hand, Actinomycetes reproduce at a slower rate than mycoparasite *T. harzianum*.

Shrestha et al. [21] also found that the *S. narbonensis* colonies were much lower than *Trichoderma*. They hypothesize, Actinomycetes develop slowly and cannot compete with generalist saprophytes and mycoparasites such as *T. harzianum*, which likely sporulate abundantly on the soil. Lahdenperä et al. [22] reported that when actinomycetes (particularly Streptomyces spp.) do not colonize plant roots in the soil, they are considered inferior competitors. However, *S. narbonensis* can colonize in extremes environments because of their physiological and biochemical adaptive abilities [23][24]. A lower number of microbial populations were noted in soil by infected *S. narbonensis* and *T. harzianum*. 1:4 treatment. The population of microorganisms experienced a very rapid increase on September 5 compared to September 2.



(a) **(b)**

Fig. 1. Fungal and Bacterial Population: (a) Microorganism growth periods, (b) Number of Microorganisms.

3.3 Biological agents as Decomposers of on marginal soils

Biota abundance is primarily determined by its food reserves, including carbon, nutrients, water, and oxygen. Of the three soil samples, andisols are rich in available P, but few other nutrients (N, C, alkaline) but also in some conditions P, in andisols are not available for the plant. Andisols contents Fe lower than P, so Available P is rich enough (Figure 1).

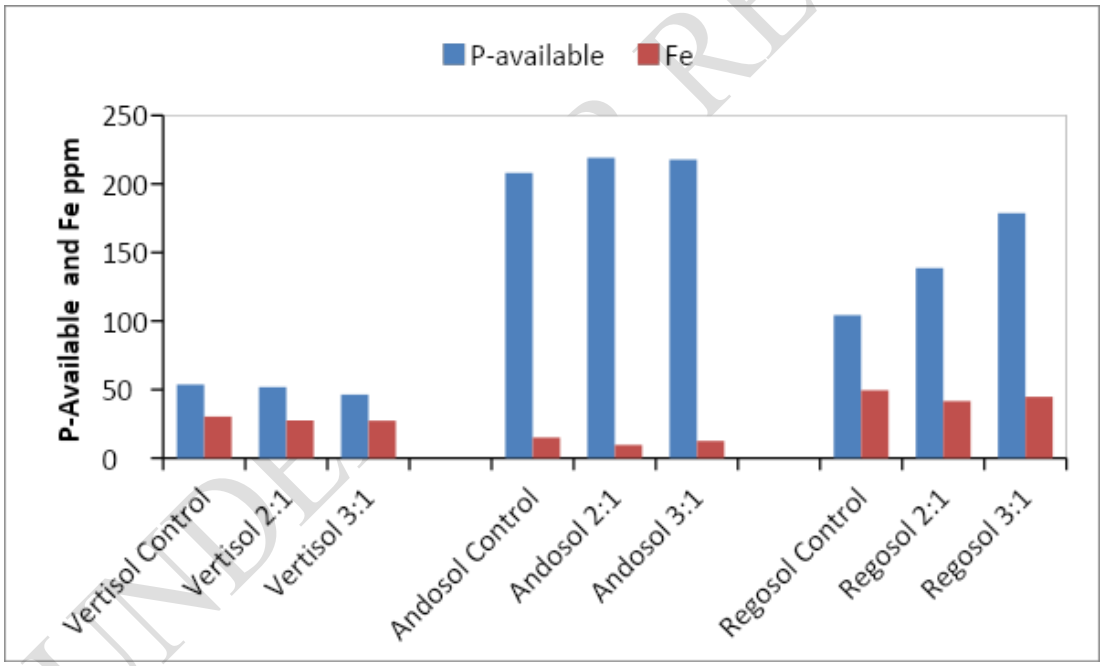


Fig. 2. P-Available and Fe Test Result

The addition of biological agents almost did not affect the nutrient content in the soil but only affected the available P regosol soil. Initially, the abundance of microbes affected the soil's organic C-decomposition. The more microbes, the lower the C-organic into mineral nutrients (Figure 2).

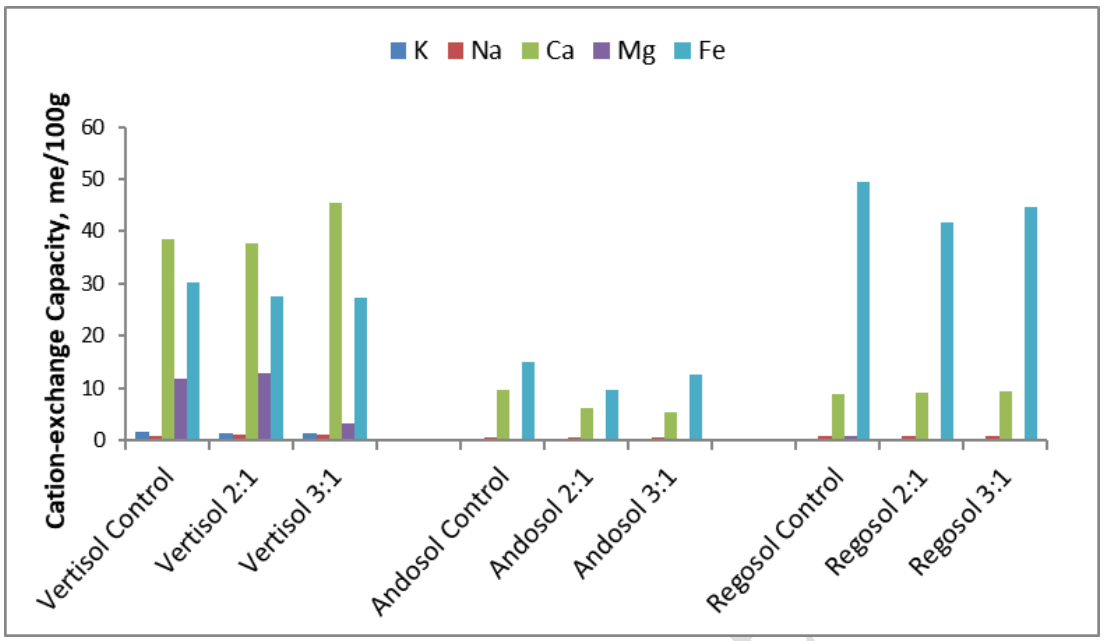


Fig. 3. Cation-Exchange Capacity Test Result

Microbes, such as beneficial fungus and bacteria found in the soil rhizosphere, are critical for nutrition provision by solubilizing organic materials [25]. Additionally, these rhizosphere microbes improve the availability of nutrients to plants, hence improving plant growth and production [11]. Our work demonstrates that the co-application of *T. harzianum* and *S. narbonensis* boosted the number of soil microbial communities compared to a control. Their interaction with the soil microbiota should be further investigated to determine any potential side effects. This was accomplished through molecular identification of fungal strains isolated from soil samples using a combination of multiplex-PCR, fingerprinting methods (rep-PCR), and tef1 sequence analysis.

The results of the analysis were visible on Andisols and Vertisols but did not appear on Regosol. This result shows that Regosol is deficient in C so that the level of decomposition and the resulting nutrients is also low. The high C-org of Vertisols, presumably due to the absence of oxygen used by microbes to decompose causes C content to remain high. Vertisols' particular characteristic is swelling when it contains water and shrinking when it is dry. Vertisols usually contain the dominant clay, so they have less porosity than others and are denser.

Gresik Vertisol soil is thought to be alkaline because the pH value is close to 7, and less than 8 has a high alkaline saturation level. Likewise, the land of Regosol Kediri. On the other hand, the soil of Andisols is slightly acidic with a high level of decomposition due to high land management causing porous land, high nutrient storage and easy washing. Microbial activity is high so that C levels decrease in line with the increase in microbes. In several studies showed that *T. harzianump.* and *S. narbonensis* act ass decomposition agents and biological agents of bioremediation [26][27].

The decomposition of organic matter in soil is determined by time as well as the presence of organic matter and the population of microorganisms. Soil analysis carried out 30 days after BCAs application showed that microorganisms did not affect soils nutrient content. It needed more time for further evaluation. Microorganisms need a suitable condition to live in these

soils with each character that may not be suitable for microorganism life. The quantity of microbial biomass-C exhibited a strong association with its turnover time, indicating that the turnover time of microbial biomass-C is a critical factor in controlling its accumulation in soil. The dark red soil had the highest turnover time of microbial biomass-C at 215 days, followed by the humic Andisol at 134 days, brown forest soil at 97 days, and light-coloured Andisol at 83 days. The regosol had the shortest turnover time at 45 days [28]. The provision of microorganisms is usually accompanied by adding organic matter in order to support the life of microorganisms and increase the source of soil nutrients. The apparent percentage of N assimilation in microbial biomass from added Microorganisms was linearly and positively correlated with the C:N ratios at maximum biomass formation and the end of incubation [29]. A holistic functional strategy is required to find and isolate more Trichoderma strains for soil health and plant yield [30].

4. CONCLUSION

Our results showed the combination of *Streptomyces* sp. and *T. harzianum* potential as PGPMs, BCAs and decomposers in marginal soil fertility of acidic soils, vertisol and regosol. Moreover, the application of these two microorganisms in soil could increase the number of leaves and plant height compared to control. *Strepomyces*sp and *Trichoderma*sp could grow in different soils and affect the available P regosol soil. Initially, the abundance of microbes affected the soil's organic C-decomposition. The more microbes, the lower the C-organic into mineral nutrients.

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