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Conference Paper

Utilization of Microorganisms *Streptomyces* sp. and *Trichoderma* sp. in Supporting Sustainable Agriculture

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ABSTRACT

Utilization of *Streptomyces* sp and *Trichoderma* sp microorganisms as biological control agents (BCAs), PGPM, bioremediation, and biofertilization are management alternatives in a sustainable agricultural system and food security. The purpose of the results compilation of this research is to examine the use of *Streptomyces* sp and *Trichoderma* sp. microorganisms in sustainable agricultural practices that support a healthier and environmentally friendly agricultural system. The *Streptomyces* sp and *Trichoderma* sp microorganisms used were the results of screening and isolation from tomato and chili fields contaminated with pesticides. Application of *Streptomyces* sp and *Trichoderma* sp with various forms of formula was carried out on shallot, tomato, and rice plants on a screenhouse and field scale. The results of this research show that single microorganisms or consortium forms of *Streptomyces* sp., *Trichoderma* sp. can control tomato fusarium wilt disease, onion moler disease, fruit flies, and rice pests, increase soil nutrient content, and increase plant production and yield, as well as the continued presence of BCAs on agricultural land.

Keywords: Biological control agents, combination, Streptomyces sp., and Trichoderma sp.

Introduction

Microorganisms play an important role in improving sustainable agriculture and ensuring food safety, presenting an environmentally friendly alternative to chemical fertilizers and pesticides. Utilizing the natural abilities of these microscopic organisms can increase soil fertility, increase plant growth, and manage pests and diseases more effectively (Bhardwaj et al., 2014). Globally, agriculturalists are switching from agrochemical practices to agro-biotechnological practices by using soil microbes as a source of fertilizers. In developed countries, soil microbial communities have been considered the prime factor for sustainable agricultural practices for the last few decades (Baćmaga et al., 2022). A microbial consortium improves the productivity of crop and soil in extreme stress conditions much better than single-strain inoculants. Therefore, microbial fertilizers and consortium are the best solution to achieve sustainable agricultural practices worldwide (Gehlot et al., 2021).

The use of microbial biological control agents (BCAs) as biopesticides, such as *Trichoderma* sp. and *Streptomyces* sp. continues to be developed in the agricultural industry. *Trichoderma* sp. is a soil fungus belonging to the Ascomycota class, this fungus is able to control pathogenic fungi such as *Fusarium* sp., *Ralstonia* sp., and *Rizoctonia* sp. (Tambunan et al., 2014). *Streptomyces* sp. is a soil Actinomycetes bacterium that can suppress the development of other microbes and act as an entomopathogen or pathogen that attacks insects. Research on insect pest control with *Streptomyces* sp. has been done to control armyworms (*Spodoptera frugiperda*) and fruit flies (*Bactrocera* sp.) with the chitinase enzyme which degrades the polysaccharide chitin during the insect's molting process (Suryaminarsih et al., 2019).

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In the aspect of plant protection, *Streptomyces* sp. and *Trichoderma* sp used as biological agents to prevent and overcome the impact of attacks by the fruit fly *Bactrocera* sp. in chili plants, Meloydogyne nematode, Spodoptera litura, Nezara Viridula in soybean, Moler diseases, Wilt of Fusarium, Yellowing Virus, Antracnose of chili. Indirectly through induction of resistance (Suryaminarsih et al., 2016; Risdiyanti et al., 2023; Agustin et al., 2023; Agadhia et al., 2022). Application straw bioamillerant combined with *Trichoderma* sp. reduced brown spot, sheath rice blight, bacterial leaf blight diseases, and increased the rice grain yield (Simarmata et al., 2016).

Based on the results of Baćmaga et al.'s research (2022), the use of field doses of the pesticide karetconazole does not affect the number and diversity of the genus *Streptomyces* spp., so that it has the potential to become a microorganism capable of bioaugmentation in pesticide-contaminated soil. According to Kumar et al. 's research (2022) *Streptomyces* spp. produces secondary metabolite compounds that help the degradation process of various pesticides. So it can be concluded that *Streptomyces* spp. has the potential to be resistant to pesticides and is effective as a biological agent. *Streptomyces* sp. and *Trichoderma* sp. can be combined in application because they have good compatibility. This was confirmed by Fitriana that *Streptomyces* sp. and *Trichoderma* sp suspensions in PGE liquid media contained these two microorganisms when isolated again (Agustin et al., 2023). Multiantagonis *Streptomyces* sp. and *Trichoderma* sp, produce beneficial compounds such as Indole-3-acetic acid (IAA) to stimulate plant root growth and siderophore compounds to stimulate nutrient absorption in the soil (Fitriana et al., 2020).

The provision of inorganic fertilizer and synthetic pesticides has led to an increase in land nutrients and production, but the environmental balance and land productivity have decreased day by day, and these agricultural cultivation activities have continued until now (Nawaal et al.,2022). *Streptomyces* sp. and *Trichoderma* sp. can act as soil bioameliorants. The combination of Streptomyces and Trichoderma, as well as several other beneficial microorganisms in rice straw compost media, can increase the efficiency of fertilizer use and can improve poor soil conditions (Kumar et al., 2022).

In developed countries, soil microbial communities have been considered a major factor in sustainable agricultural practices over the past decades. The activity and interaction of soil microorganisms have been proven to encourage plant growth, soil quality, and productivity, as well as a biopesticide to control plant pests and diseases. *Streptomyces* sp. and *Trichoderma* sp are soil microorganisms that can be used as bio pesticides, bio fertilization, growth regulators, and bio augmentation/bioremediation. Based on the abilities of this microorganism, the study of *Streptomyces* sp. and *Trichoderma* The use of these microorganisms for sustainable modern agriculture to achieve food security, but also has obstacles that need to be overcome through further research.

Material and Methods

Isolation of biological agents and pathogens

The material for isolating biological agent microbes is the soil of chili fields and tomato fields in Pare-Kediri village that uses pesticides intensively. Isolation of biological agent microbes, using the soil plating method by Dhingra and Sinclair (1994): Soil from chili fields and tomato fields was weighed with an analytical balance in the amount of 1 gram, then a suspension was made with a dilution of 10-4. Next, 1 mL was taken aseptically and spread evenly into the GNA medium in a petri dish for isolation of *Streptomyces* sp. Isolation of *Trichoderma* sp. was carried out like Streptomyces isolation, but the isolation medium was PDA. The biological agents obtained were then purified and multiplied on PDA media in Petri dishes and then tested for antibiosis.

The method used to isolate *F. oxysporum* was stimulation of spores from fresh material (Sastrahidayat, 1994), the pathogen was isolated from the stems of tomato plants from the field

(Wajak-Malang District) that were attacked by Fusarium wilt. The diseased part of the plant stem is cleaned, then sterilized with 70% alcohol, then air-dried, and then the skin is cut with a scalpel. The incision was then inoculated in PDA medium. The pathogenic fungi that grow are isolated and purified, then the purified F. oxysporum fungus is propagated on PDA media.

Antagonist and identification testing

Streptomyces sp., was identified at the Tropical Diseases Center (TDC) Airlangga University using the 16S rRNA DNA sequencing method. Trichoderma sp was identified at IPB using the 18S rRNA sequencing method. Fusarium sp was identified using the Taxonomy book and Koch's postulate method.

Antagonist testing was a preliminary test carried out to determine biological agents against pathogenic fungi. The media used in the antagonist test was PDA media with a double culture method. The 7-day F. oxysporum fungus culture was cut using a 5 mm diameter cork borer and placed opposite the Streptomyces sp culture. 14 days old. Then incubate and observe its development for 7 days.

Compatibility test

Descriptive and parametric research steps for compatibility testing of biological agents *Streptomyces* sp., and Trichoderma sp. In general, it consists of the preparation of colonies of biological agents, compatibility tests, control of each biological agent, and observations. Observations include: Colony growth patterns overlap, there are no clean zones between biological agents (non-antibiosis), the diameter of the compatibility treatment colony is greater than or equal to the diameter of the control.

Data from observations of the estimator parameters, namely the inhibitory power of biological agents, were analyzed using analysis of variance (F Test) with a Completely Randomized Design (CRD). If the treatments are significantly different, a difference test is carried out using the Duncan test. Data analysis using SPSS 18 tools.

Preparing of biocide

Propagation of Trichoderma sp. and Streptomyces sp. in ECG media (Sugar Potato Extract), each combined isolate treatment (Trichoderma sp. and Streptomyces sp.) was carried out by taking 4 plugs each using a cork drill (each with a diameter of ± 8mm). Multiplication isolate Trichoderma sp. and Streptomyces sp. in culture media, ECG is carried out by taking 2 samples each using a cork drill (each with a diameter of ± 8mm). Then, all treatments were inoculated into 150 ml of ECG (Potato Sugar Extract), GN (Glucose Nitrate), and Coconut water culture media in a 250 ml capacity Erlenmeyer flask and shaken using a shaker at 120 rpm at room temperature for 14 days (Widiantini et al., 2018).

Prepare the composition of the microorganism *Streptomyces* sp. and *Trichoderma* sp. with a ratio of 3:1 on sugar potato extract (EKG) media by boiling 3 liters of water. Boil the potatoes until they change color and are softer. Then drain the potatoes; Add 17 liters of water to the potato juice, then boil until it boils. Put the finished media into a sterile gallon and close tightly. Add Streptomyces sp isolate. And Trichoderma sp. into the cooled medium after 24 hours; Assembling gallons of media with fermenters to help metabolize microorganisms in ECG media.

Biocide application

Biocide application was carried out in 2 stages in several studies:

1. Screen house scale on tomato plants. In vivo testing was carried out by inoculating a suspension of the *Fusarium* sp fungus. 107 spores/ml in planting media in 25 cm polybags. The initial stage of planting begins with the application of biocide by pouring 10 ml into the planting hole that has been applied with patogen. Applications are also given once every two weeks, 10 ml per polybag. Plants are maintained by watering them every day.

2. Field scale: Practice for application, 20 L of water plus 2 glasses of *Streptomyces* sp. and 1 glass of *Trichoderma* sp., on rice field tomatoes, shallots. Two lands had Biocide combined with vermicompose and humate acid.

Results and Discussion

Isolations, identifications, Biological Control Agents (BCA), and Pathogen

Streptomyces sp. isolated from chili fields on PDA media had colonies in yellow, bright red, and white, like cotton fibers, not shiny. The results of the Gram staining test showed that the isolate was Gram-positive. Microscopic observation of this bacterium showed a slender hyphae morphology of 11 μm, branching without partitions. The spore chains were circular and elongated 17.61 x 41.8, the spores were round, hyaline, with a diameter of 11.67 - 12.10 μm. Basic Local Alignment Tool (BLAST) analysis showed that the majority of bacteria from DNA sequencing were in the Streptomyces griseorubens species area, with a total value of 603 approaching 97% similarity. The identification of Actinomycetes spp. by DNA sequencing showed that Actinomycetes isolates from tomato land are closely related *Streptomyces narbonesis*,

Test of the antagonistic ability of Streptomyces griseorubens f.sp. capsicum and Streptomyces narbonensis against the pathogenic fungus F. oxysporum showed that it could inhibit the development of the pathogenic fungus on PDA media, in Petri dishes. Isolation of biological agents *Trichoderma* sp. can inhibit the development of colonies of the pathogenic fungus *F. oxysporum*. BLAST analysis of DNA sequencing results with 18S rRNA showed a level of similarity with Trichoderma sp. 90%. Microscopic observations that have been carried out on pathogenic fungi (fusarium wilt) show similar characteristics with *F. oxysporum* f. sp. lycopersici. namely, single-celled, colorless, oval or ovoid microconidium, and coil-shaped, colorless macroconidium, mostly with more than two partitions. The Koch Postulate Test showed that tomato plants inoculated with F. oxysporum isolates showed the same symptoms as tomato fusarium wilt disease caused by *F. oxysporum* f. sp. lycopersici.s).

Compatibility of Streptomyces sp. and Trichoderma sp.

Descriptive observation results of the compatibility of biological agents *S. griseorubens.,* and *T. harzianum* grown together on PDA media, 14 days after inoculation showed no antagonism between biological agents, this can be seen from the absence of a clean zone (the presence of antibiosis), *T. harzianum* colonies compared to *S. Griseorubens* are wider and grow denser, greener, *S. griseorubens* grows slower, but tends to be higher and taller'. Table 1 shows the average growth diameter of *S. griseorubens* colonies in the biological agent compatibility test (ST) compared to the control (SS), days 2 and 4, after inoculation (dai), larger and significantly different. Overall, from day 2 to day 8, the growth diameter of *S. griseorubens*. colonies in the compatibility test (ST) and control (SS) increased.

Table 1. Growth average of *Streptomyces* sp. on compatibility test dan control

Treatment	Average of colony diameters (…cm) days after inoculations (dai)			
	2 dai	4 dai	6 dai	8 dai
Compatibility ST	0,800 a	0,980 a	0,980 a	1,360 a
Control SS	0,660 b	0,860 b	0,980 a	1,680 a
t-tes	0,045	0,033	0,089	0,078

Explanations: ST was colony of *Streptomyces* sp., and *Trichoderma sp,* SS was a 2 colony of *Streptomyces* sp. Different letters next to numbers in one column indicate a significant difference. (ρ < 0,05)

Biopesticide in screen house scale

The average severity of fusarium wilt disease in the administration of biological agents *Streptomyces griceurubens.*, and *Trichoderma harzianum* was significantly different and smaller than the average severity of fusarium wilt disease in the control (Table 2).

Table 2. Severity average of Fusarium wilt diseases of the tomato plant

No	Applications of BCAs	Severity of average diseases (%)			
		41 dai	48 dai	55 dai	62 dai
1	Control (Without BCAs) (C)	3,80 a	7,60 a	17,86 a	44,64 a
2	*T. harzianum.* (ST)	0,00 a	5,70 a	7,14 a	19,65 b
3	*S. griseorubens* (S)	0,00 a	1,90 a	15,42 a	16,07 b
5.	*S. griseorubens,* *T. harzianum* (ST)	1,52 a	5,77 a	5,77 a	14,29 b

Note: Dap was days after planting. The same letter next to the number in the same column indicates that the Duncan test results are not significantly different ($p < 0.05$)

Applications of combinations of *Trichoderma* sp and *Streptomyces* sp (3:1) in EKG media can reduce the gall of the meliodogyne nematode of the cherry tomato plant.

Table 3. The effect of combination treatment of Trichoderma sp. and Streptomyces sp. in various production media on the number of gall/gram of cerry tomato plant roots

No.	Treatment	Count of gall/gram
	Trichoderma sp. and *Streptomyces* sp.	
1.	Control	73,93 c
2.	*Trichoderma* sp.	21,00 b
3.	*Streptomyces* sp.	23,31 b
4.	*Trichoderma* sp. and *Streptomyces* sp. 2:2	13,54 ab
5.	*Trichoderma* sp. and *Streptomyces* sp. 1:3	9,23 a
	Production Media	
1.	*Potato Sugar Extract (EKG)*	24, 5 a
2.	*Glucose Nitrate*	28,56 b
3.	*Coconut Water*	31,58 c

Note: The same letters next to numbers indicate no significant difference ($\alpha < 0.05$)

On land given biological agent *S. griseorubens, T. harzianum* produced higher fruit production compared to fruit production on land not given biological agent. In Table 4, it can be seen that the average fruit production in tomato plants given biological agents gave the same results, and was higher and significantly different from fruit production in plants without biological agents. This higher fruit production is because biological agents can increase plant growth and inhibit the development of Fusarium wilt disease.

Table 4. Average tomato fruit production with the provision of biological agents on tomato seedlings planted in soil containing the pathogen *F. oxysporum* .

No	Applications of BCAs treatment	Average fruit weight	
		gram/plant	Transf $\sqrt{(x + 0{,}5)}$
1	*S. griseorubens,* (S)	205.0000	14,0014 a
2	*S. griseorubens, T. harzianum,* (ST)	200.000	13.8674 a
3	*T. harzianum* (T)	147.000	12.0275 a
5.	Control (Without BCAs) (K)	25.000	4.4907 b

Note: The same letters next to numbers indicate no significant difference ($\alpha < 0.05$)

Practice for application of biosida at the field scale

Application of the biological agent *Streptomyces* sp. and *Trichoderma* sp. can reduce the number of individual pests and natural enemies of predators that remain, and even increase so that they can suppress the presence of pests. All types, roles, and numbers that have been observed in rice planting with biological agent application treatment and without biological agent application treatment were carried out in Mojotengah village, Menganti District, Gresik Regency. Presented in Table 5.

Table 5. BCAs Applications *Streptomyces* sp. dan *Trichoderma* sp at rice planting Mojotengah village

Kind of Insect			Insect Status	∑Population of Insects	
Ordo	**Famili**	**Genus/ Spesies**		**Without BCAs**	**With BCAs**
Lepidoptera	Crambidae	*Cnaphalocrocis medinalis*	Insect Pest	200	50
Orthoptera	Acrididae	*Valanga nigricornis*	Insect Pest	50	25
Hemiptera	Alydidae	*Leptocorisa aculata*	Insect Pest	90	40
	Delphicidae	*Nillaparvata ligens*	Insect Pest	300	100
Diptera	Muscidae	*Atherigona oryzae*	Insect Pest	125	55
Rodentia	Muridae	*Rattus argentiventer*	Insect Pest	10	10
Hemiptera	Veliidae	*Microveliadouglasi atrolineta*	Predator	30	70
Hymenoptera	Sphecidae	*Sphecidae sp.*	Predator	100	300
Coleoptera	Coccineliidae	*Harmonia octomaculata*	Predator	50	95

Application of biocide *Streptomyces narbonensis* , *Trichoderma harzianum* with the addition of humic acid to tomato, melon, and chili plants at marginal land can increase fruit production and reduce plant pest attacks. The average fruit production of melon, chili, and tomato plants with the application of multiantagonists and humic acid was higher than the average fruit production without the application of multiantagonists and humic acid (Table 6).

Table 6. Average productions of tomato, chilli, and melo at Pare

BCAs dosage	chili(gr)	tomato(gr)	melo(Kg))
100 ml/plant	1182.86	9472.33	182.86
200 ml/plant	1262.61	9263.33	262.61
300 m/plant	1235.04	9885.33	235.04
300 ml of water/plant	60.53	4648.33	15.40

Streptomyces as a Plant Growth Promoting Bacterial (PGPB) is known to trigger plant growth through the production of indole-3-acetid acid (IAA) which plays a role in root growth, producing siderophores to increase the availability and absorption of nutrients in the soil (Damam et al. 2016), as well as produces extracellular proteases (Palaniyandi et al., 2013) and antibiotics (Palaniyandi et al., 2013) which are useful in inhibiting the growth of plant pathogens. Apart from that, Streptomyces has another interesting property, that is able to produce VOCs (volatile organic compounds) which play a role in plant growth and defense. Several types of VOC compounds that have been detected from *S. alboflavus* TD-1 are 2-methyl isoborneol, a-cubebene, 1H-Indene,1-ethylideneoctahydro-7a-methyl-(1Z,3a, alpha,7a, beta), H-Indene, 1-ethylideneoctahydro-7a-methyl-, cis-, and geosmin (Wang et al., 2013).

The combination of *Streptomyces* and *Trichoderma* as well as several other beneficial micro-organisms in rice straw compost media can increase the efficiency of fertilizer use and can improve poor soil conditions. The results of soil analysis from shallot fields that have been given biological agents, and moler disease control using fungicides showed the status of the development of available C, N, P, K content (Table 7).

Table 7. The shallot field soil status after applications of Biocide *Streptomyces* sp. and *Trichoderma* sp.

No	Parameters	Status		
		Soil condition	Without BCAs	BCAs and Fungicide
1.	pH	6.1	6.0	6.1
2.	C	1.19 %	1.27 %	2.03 %
3.	N	0.09 %	0.12 %	0.18 %
4.	P	22 ppm	23 ppm	27 ppm
5.	K	0.53 me/100g	0.55 me/ 100 g	0.55 me/ 100 g

In general, soil acidity (pH) given to Streptomyces and Trichoderma does not increase or decrease pH. Meanwhile, the nutritional availability of Streptomyces and Trichoderma can increase the content of C-oranic, N-total, P-available, and K-available. Giving Streptomyces and Trichoderma treatment there was an increase in organic C, indicating that there was a breakdown of organic material by microorganisms in this treatment, so that the availability of organic C increased due to an increase in the breakdown of organic material by microorganisms (Mindari & Purnomo, 2011; Suryaminarsih et al., 2017).

The result measurements of C organic in the soil after giving treatment *Streptomyces* sp, vermin compost, and synthetic fertilizer Ponska show the provision of organic fertilizer vermin compost did not show a significant difference in C. organic, but the maximum concentrations of Ponska were 75 % better than only Ponska (Figure 1).

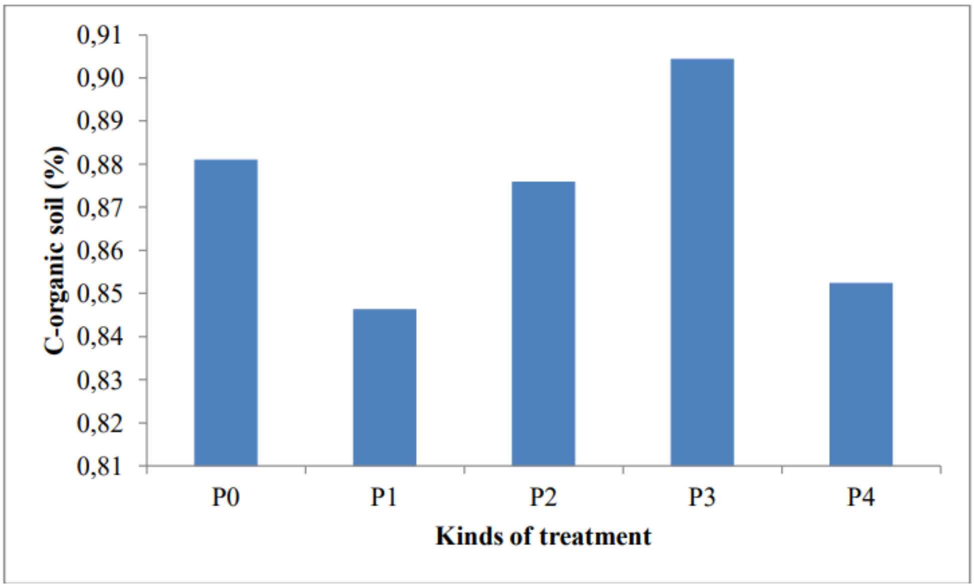

Figure 1. Histogram of C-organik

Conclusion and suggestions

The conclusion drawn from this discussion is that Streptomyces and Trichoderma are multirole microorganisms and beneficial for agricultural businesses to support food security through modern, sustainable agriculture.

Microbial reactions are relatively slow and have a low shelf life. It can be overcome by selecting microorganisms and making appropriate formulations of biological agents, as well as using more modern biotechnology to increase the effectiveness of these microorganisms. Further research is needed to explore other potentials of *Streptomyces* sp. and *Trichoderma* sp., as well as more practical and efficient production.

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