

Incorporating adoption of agricultural bio-innovation in a famers' participatory parental seed production

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Abstract. TRIBAS is a biopesticide formulation developed from bacterial consortia of *B. subtilis* isolates. The formulation can control maize diseases, particularly those caused by pathogenic fungi. This research was conducted to validate the effectiveness of TRIBAS to support growth and yield of several maize inbred grown for parental seed production of several high yielding hybrids. A local seed growers' participatory evaluation was set up in three districts in South Sulawesi (Maros, Soppeng, and Bone) using 5 maize inbred, i.e., MR-15, N-79, NEI-9008, AMB-20, and MAL-03. Each was grown following standard seed growing procedures with an additional treatment, with and without TRIBAS application. The results showed that the application of TRIBAS through seed treatment and plant spraying was able to inhibit the incidence of three main fungal diseases in maize, i.e, *P. philippinensis*, *R. solani* and *B. maydis*. The application did not result in significant seed productivity increase; however, it provides economic added value of up to IDR 5,724,000/Ha, considering that the seeds being propagated are parental seeds whose prices are relatively high (Rp 90,000/Kg). Continuous application of TRIBAS is expected to increase bacteria population in the soil, which, gradually would result in the increase of crop productivity.

1 Introduction

Antagonistic microorganisms such as *Bacillus subtilis* can be formulated to produce a pest control for biological product. Beside preventing yield losses through controlling pests and diseases, biopesticides is also beneficial for environmental and human health by contributing in reduction of chemical pesticides which results in safer quality of agricultural products [1]. Biopesticides are important components in integrated biological control which had been

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proven in reducing the risk of resistance of plant pests towards chemical pesticides [2], conserving natural enemies, maintaining environmental health and also safer for health [3].

One of *B. subtilis* isolate, TM4, is known to be virulent as biological agent of maize plant disease. Previous study which tested this bacterium in vitro has shown its ability to reduce the development of *Bipolaris maydis*, *Rhizoctonia solani* and *Fusarium moniliforme* by using a double culture method namely diffusible toxic metabolite test and volatile compound test [4-5]. The formula of antagonistic bacteria *B. subtilis* TM4 was effective in reducing the development of leaf blight and leaf blight disease through seed treatment [6]. Furthermore, there are also *B. subtilis* BNt8 and TM3 isolates which able to reduce the development of *Fusarium moniliforme* fungi and support seed development [7]. In particular, the application of this bacterial isolate was also reported to have a high reproductive ability, shown by the high number of bacterial colonies in the root sprout which seeds treated with *B. subtilis* BNt8. Application of *B. subtilis* BNt8 formulation can suppressing leaf blight up to 13% and potentially increasing yields by 26% [8].

TRIBAS is a bacterial consortia of *B. subtilis* TM4, BNt8 and TM3 isolates which were combined with vegetable material. This formulation has ability to control diseases of maize plants, particularly the disease which caused by pathogenic fungi [4-5]. As well as suppressing the growth of pathogens, TRIBAS is also PGPR (Plant Growth Promoting Rhizobacteria) which can stimulate plant growth. Several studies recommend to apply TRIBAS in combination with resistant varieties due to optimizing the result. As a one of bio-innovation developed by Indonesian Agency for Agricultural Research and Development (IAARD), the characteristics of TRIBAS which are cheap and environmentally friendly can be used to increase the productivity of maize seeds, which so far are still relatively low [8,9,10].

Previous study showed that the use of TRIBAS as biopesticide does not necessarily increase the productivity of seed yields in the first year of use. When applied to seeds, the bacteria *Bacillus subtilis* contained in TRIBAS will colonize the root system of plants, so these bacteria can reduce the development of various plant diseases [6, 11, 12]. These bacteria continue to live in the root system of plants and can protect plants throughout the growing season. As an innovation product, TRIBAS must be disseminated to local champions farmer who have ability as a seed grower and complete the criteria in producing seeds. Farmer assistance from research institution is a must so that the quality and purity of the seeds produced is maintained. In addition, the continuity of assistance, especially in the application of TRIBAS, is important to know the resulting increase in productivity can be known.

This research was conducted to determine the effect of biopesticides application in increasing growth and yield of several maize lines as well as its economic advantages through the participation of local seed growers.

2 Materials and methods

2.1 Study area

This study was conducted in South Sulawesi Province, Indonesia in dry season 2019. This study was performed two stages, the first was preparing the biopesticide formula were conducted in Plant Protection Laboratory, Indonesian Cereals Research Institute (ICERI) in Maros district and the second was field experiment were conducted in farmer's land in three districts, i.e., Maros, Soppeng, and Bone. The farmers involved were local champions in those areas. Those considered as local champions could be farmers or seed growers who have been participating in ICERI research projects, including those with sufficient knowledge and skills in seeds production.

2.2 Procedures

2.2.1 Preparation of TRIBAS biopesticide formula

Three isolates of antagonistic bacterial *B. subtilis* namely, TM3, TM4 and BNt8 (ICERI collection) were propagated in Nutrient Agar (NA) culture medium then incubated for 72 h room temperature. The harvested bacterial isolates were inoculated into Nutrient Broth (NB) medium and incubated at room temperature for 48 h on a rotary shaker for 48 h and then centrifuged at 5000 rpm. The obtained supernatant was removed and leaved about twenty percent of sedimented substances. The substances of bacterial mass, the results were mixed into the carrier materials in the form of talc, yeast extract, carboxymethyl cellulose, and Arabic gum. Subsequently, the formula was dried on a sterile worktable tray for 72 h and then mashed and finally stored in a sterile container at room temperature. The *B. subtilis* biopesticide formula was then ready to be applied in the field. This formulation was finally defined as a TRIBAS biopesticide formulation [5-6].

2.2.2 TRIBAS Efficacy Field Test

Five maize inbred lines namely MR-15, N-79, NEI-9008, AMB-20, and MAL-03 were planted in farmers' land with an area of 2.0 Ha for each maize inbred, consisted of two treatments: with and without TRIBAS application. TRIBAS was applied for 4 times: at planting as seed treatment with a dose 8 g/kg of seed and at 15, 30, and 45 days after planting (DAP) through spraying with a dose 3 g/l solution. Synthetic chemical pesticides were not applied at all for both treatments [6].

The observations were focused on the intensity of the diseases and yield components. The first parameter was observed by calculating the percentage of disease attack and the severity of attack focused on the natural attack of *B. maydis*, *P. philippinensis*, and *R. solani* following the Ajuha and Payak scoring method [13]. Meanwhile, the yield components was observed for number of rows, number of seeds per row, ear diameter, ear length, seed yield, weight of 1000 seeds, and productivity of seed yield (dry shelled), following the CIMMYT manual yield trial [14].

2.3 Data analysis

The data were analyzed as a completely randomized design and means were separated using Fisher's protected least significant difference (LSD). Each data represented were means of three replicates. To compare the disease severity in different treatments, pair sample *t*-test was performed. The yield components were analyzed by Tukey's Multiple Comparison Test. The test results were then analyzed from financial aspect to determine the advantages of using TRIBAS biopesticide from the economic aspect. Financial analysis was analyzed by calculating the delta of production quantities with and without TRIBAS. Then the delta is multiplied by the seed price which is assumed to be 90,000/kg. Financial analysis can be seen from the following equation:

$$TR = P \times (Q_1 - Q_0) \tag{1}$$

Where:

TR = Total Revenue (Rp)

P = Seed Price (Rp/kg)

Q₁ = Total Production with TRIBAS (kg)

Q₀ = Total Production without TRIBAS (kg)

3 Result and discussion

There was a difference in the response of maize inbreds resistance to downy mildew *P. philippinensis*, stem and leaf blight *R. solani* and *B. maydis* between two different treatments, with and without biopesticide. Studies revealed that all maize inbreds treated to biopesticide tend to have a better resistance to major diseases of maize compared to non-treated biopesticide. This could be seen by the number of damaged leaves caused by downy mildew, stem and leaf blight were lower.

The result of TRIBAS biopesticide examination to major diseases in maize showed differences infection level between maize inbreds. There was no significant different between the two treatments on the developing of downy mildew. There was also found no infection at all on NEI-9008 and MAL-03 maize inbreds. The incidence of downy mildew at 30 DAP indicated the highest incidences in AMB-20 maize inbred, in which without biopesticide treatment had higher (60%) incidence of this disease compared to with biopesticide treatment (50%) (Table 1). Whereas, until 60 DAP the symptom of leaf and stem blight was only found in NEI-9008 maize inbred. However, the treatments of biopesticides significantly affected on incidence of this disease, in which biopesticide treatment had a lower incidence (25%) of leaf and stem blight compared to without biopesticide treatment (40%) (Fig.1).



Fig. 1. The symptom of downy mildew in AMB-20 maize inbred (left); stem and leaf blight in NEI-9008 maize inbred (middle); and leaf blight in MAL-03 maize inbred (right).

Table 1. Average of downy mildew *P. philippinensis* and stem and leaf blight *R. solani* disease incidence of 5 different maize inbreds.

Maize Inbreds	% downy mildew at 30 DAP		CV (%)	% stem and leaf blight at 60 DAP		CV (%)
	TRIBAS	No TRIBAS		Tribas	No Tribas	
	MR-15	25 ^a	30 ^a	6.8	0 ^a	0 ^a
N-79	35 ^a	40 ^a	11.8	0 ^a	0 ^a	-
NEI-9008	0 ^a	0 ^a	-	25.3 ^a	40.1 ^b	22.1
AMB-20	50 ^a	60 ^a	7.8	0 ^a	0 ^a	-
MAL-03	0 ^a	0 ^a	-	0 ^a	0 ^a	-

Application of biopesticide formula can induce resistance of maize seeds towards leaf blight infection (Table 2). The treatment of biopesticide tended to significantly lower than without biopesticide. At the age of 60 DAP, there was a significant different of the lowest difference of disease incidence between with (23.3%) and without (42.5%) biopesticide treatments found in N-79 maize inbred line.

Table 2. Average of downy mildew *B. maydis* disease intensity of 5 different maize inbreds.

Maize Inbreds	Intensity of downey mildew (%) at-					
	30 DAP		45 DAP		60 DAP	
	Tribas	No Tribas	Tribas	No Tribas	Tribas	No Tribas
MR-15	9.0 ^a	13.3 ^b	9.5 ^a	21.1 ^b	21.7 ^a	25.1 ^b
N-79	7.8 ^a	11.3 ^b	22.3 ^a	38.5 ^b	23.3 ^a	42.5 ^b
NEI-9008	11.7 ^a	28.5 ^b	18.7 ^a	36.7 ^b	37.0 ^a	39.5 ^b
AMB-20	11.2 ^a	13.1 ^b	31.2 ^a	31.3 ^a	34.5 ^a	34.4 ^a
MAL-03	10.4 ^a	16.4 ^b	41.1 ^a	45.8 ^b	59.1 ^b	54.5 ^a

Similar letter in the column at the same time of observation indicates non-significant at LSD 5%.

Applying biopesticide affected plant growth and stomatal density (Table 3). There was a significant effect of biopesticide treatments on plant height of N-79, NEI-9008, AMB-20 and MAL-03 maize inbreds. Applying biopesticide increased plant height all maize inbreds considerably compared to non-biopesticide treatment, except for MAL-03 maize inbred, which its plant height was higher for non-biopesticide (144 cm) compared to biopesticide treatments (133.9 cm). Conversely, applying biopesticide was not affected on stomatal density for all maize inbreds examined. Except for MAL-03 maize inbred, applying biopesticide treatment had higher stomatal density (75/mm²) compared to non-biopesticide treatment (65.4/mm²).

Table 3. Average of plant height and stomatal density of 5 different maize inbreds

Maize Inbred	Plant Height (cm) at 60		CV (%)	Stomatal Density (/mm ²)		CV (%)
	DAP			at 30 DAP		
	Tribas	No Tribas		Tribas	No Tribas	
MR-15	137.8 ^a	137.2 ^a	11.8	55.6 ^a	54.7 ^a	27.3
N-79	170.5 ^a	153.1 ^b	12.8	48.6 ^a	47.8 ^a	17.5
NEI-9008	174.5 ^a	144.2 ^b	9.0	56.6 ^a	55.7 ^a	33.5
AMB-20	156.6 ^a	135.9 ^b	9.4	42.6 ^a	44.3 ^a	27.4
MAL-03	133.9 ^b	144.0 ^a	8.4	75.0 ^a	65.4 ^b	18.7

Similar letter in the column at the same time of observation indicates non-significant at LSD 5%

The average of chlorophyll content was significantly affected by biopesticide treatments. Maize plants that were applied biopesticide treatment tend to have higher chlorophyll content compared to non-biopesticide treatment, particularly at 30 DAP. However, at 45 DAP, N-79 and NEI-9008 maize inbreds had higher chlorophyll content account for 37.5 g/ml and 38.1 g/ml respectively for non-biopesticide treatment, whereas for biopesticide treatment had lower chlorophyll content account for 35.2 g/ml and 36.1 g/ml respectively (Table 4).

Table 4. Average of leaf chlorophyll content of 5 different maize inbreds.

Maize Inbreds	Leaf chlorophyll content (g/ml)			
	30 DAP		45 DAP	
	Tribas	No Tribas	Tribas	No Tribas
MR-15	41.1 ^a	40.6 ^a	45.2 ^a	38.1 ^b
N-79	37.8 ^a	39.4 ^a	35.2 ^b	37.5 ^a
NEI-9008	38.2 ^a	32.1 ^b	36.1 ^b	38.1 ^a
AMB-20	38.5 ^a	35.1 ^b	37.4 ^a	35.0 ^b
MAL-03	34.3 ^a	32.4 ^b	54.4 ^a	37.7 ^b

Similar letter in the column at the same time of observation indicates non-significant at LSD 5%.

The analysis of variance on actual yield and yield components (total rows, total kernels per row, ear diameter, ear length, kernel yield, and thousand kernel weight) showed significant differences in the variety sources of all parameters (Table 5). Based on the maize inbreds observed, there were significant differences for all parameters except kernel yield. Meanwhile, based on the TRIBAS biopesticide application, there was significant difference on ear diameter, ear length and kernel weight, while no significant for the other parameters.

Table 5. The analysis of variance on actual yield and yield components.

Variety Sources	Degree of Freedom	Yield Components					Actual Yield
		Total Rows	Total Kernels per Row	Ear Diameter	Ear Length	Thousand Kernel Weight	
Inbreds	4	4.38**	124.88**	0.81**	16.88**	67680.88**	4037597.88**
Biopesticide	1	0.13 ^{ns}	4.03 ^{ns}	0.33**	1.63*	13146.13*	14388.30 ^{ns}
Inbreds x Biopesticide	4	1.38**	6.12 ^{ns}	0.11**	1.24*	2606.88 ^{ns}	430085.71**
Error	20	0.33	2.87	0.03	0.36	3175.10	9416.30
Total	29						
Mean		13.27	26.43	3.88	13.05	283.40	855.90
CV (%)		4.35	6.41	4.45	4.57	19.88	11.34

Furthermore, based on the interaction between inbreds and TRIBAS application, there was significant effects on total rows, ear diameter, and actual yield; significant effects on ear length; while no significant effects on the other parameters. Based on the analysis of variance on yield, in general, inbreds had more significant effects on plant appearance than the TRIBAS biopesticide application. Therefore, the effects of the interaction between inbreds x TRIBAS application was not found on all parameters.

A more detailed review of each inbred performance on each yield and yield components showed that the TRIBAS application did not always give higher values than the control (without TRIBAS), except for the kernel yield, where all inbreds showed higher values in the TRIBAS application treatment (Table 6). Meanwhile, total rows, total kernels per row, ear diameter, ear length, thousand kernel weight, and actual yield showed higher values in the treatment without TRIBAS application. However, in terms of the total mean, all inbreds showed that only in total rows, TRIBAS application did not give higher yield than without TRIBAS application. The yield advantage of the TRIBAS application over control (without the TRIBAS application) ranged between 2.3%-15.9%, as in the following parameters namely, 2.3% of total kernels per row; 3.6% of ear length; 4.1% of kernel yield; 4.8% of ear diameter, 5.2% of actual yield, and 15.9% of thousand kernel weight. Thus, the TRIBAS application had not provided a significant increase in most yield components and yields productivity.

Table 6. The effect of maize inbreds on yield components

Inbreds	Yield Components	
	Total kernels per row	Thousand Kernel Weight
AMB-20	29.00a	162.83c
MAL-03	21.83b	285.33b
MR-15	30.83a	271.67b
N-79	29.33a	453.33a
NEI-9008	21.17b	243.83b

Based on the correlation analysis (Table 7), plant height was the only growth parameter with a positive correlation with actual yield. The incidence of *P. philippinensis*, *R. solani* and

B. maydis as well as stomatal density and chlorophyll content showed a negative correlation with actual yield.

Table 7. Correlation between actual yield with disease incidence and growth parameters.

Parameters	PP	RS	BM	Plant Height	Stomatal Density	Chlorophyll Content	Actual Yield
PP	1.00	-0.54	-0.51	-0.02	-0.83	-0.41	-0.13
RS		1.00	0.06	0.25	0.07	-0.18	-0.06
BM			1.00	-0.23	0.66	0.43	-0.08
Plant Height				1.00	-0.34	-0.51	0.57
Stomatal Density					1.00	0.74	-0.25
Chlorophyll Content						1.00	-0.24
Yield Productivity							1.00

PP = *P. philippinensis*, RS = *R. solani*, BM = *B. maydis*

Applying TRIBAS for one season only as in the study on five maize inbreds resulted in an average yield increase up to 64 kg/ha, equal to Rp 5,724,000/Ha (following the present parental seeds price of Rp 90,000/kg) (Table 8). This result can be a positive stimulus for more seeds growers to apply TRIBAS, because similar studies has shown that the continuous applications of TRIBAS increased yield productivity up to 26% with the profit delta of more than Rp 15 million/Ha [6,8,15].

Table 8. Profit increase for each parental seed after applying TRIBAS

Maize Inbred	Yield (kg/ha)		Yield Delta (kg/ha)	Profit Increase (Rp/Ha) *
	TRIBAS	NON TRIBAS		
MR-15	554	411	143	5,148,000
N-79	1,859	2,691	(832)	(29,952,000)
Nei-9008	1,152	514	638	22,968,000
AMB-20	337	181	156	5,616,000
MAL-03	487	274	213	7,668,000
Average			64	5,724,000

*Seed price Rp 90,000/kg

4 Conclusion

Studies revealed that all maize inbreds treated with biopesticide TRIBAS tend to have a better resistance to major diseases of maize compared to non-treated ones. Statistically, the TRIBAS application for one season had not provided a significant increase in most yield components and yields. Based on financial analysis, the average yield after applying TRIBAS increase of 64 kg/ha, equal to Rp 5,724,000/Ha added profit. Continuous application of TRIBAS is expected to increase bacteria population in the soil, which, gradually would result in the increase of crop productivity.

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