Standardized Organic Fertilizer Formulations and their Effectiveness in Enhancing the Role of Biological Agents to Increase Disease Resistance and Maize Productivity in Marginal Lands

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ABSTRACT

Background: The importance of standardized organic fertilizers with relatively balanced levels of NPK elements, in order to increase the role of organic fertilizers as a source of nutrition for plants which at the same time can improve soil physical, chemical and biological properties and can increase the role of organic fertilizers as a medium for growth and development of biological agents so that they can increase its role as a biopesticide and biofertilizer, thereby simultaneously increasing crop productivity on marginal land.

Methods: This research was conducted in the period June-November 2019 at South Konawe Regency Southeast Sulawesi Indonesia and in the Integrated Laboratory of Plant Protection Department, Faculty of Agriculture, Kendari Halu Oleo University. Assessing and formulating organic fertilizers by taking into account the composition of the ingredients which include; C / N ratio of the total material, elemental content of NPK, C-Organic content, moisture content and pH, to produce standardized organic fertilizers, with relatively balanced levels of NPK elements. The field research was arranged in a randomized block design consisting of 3 treatments including B_0 = Inorganic fertilizer (control); B_1 = Organic fertilizer formula A + agens hayati Biofresh; B_2 = Organic fertilizer formula B + agens hayati Biofresh. Each treatment was repeated 3 times, which in total there were 9 experimental units.

Result: The formulation of organic fertilizers by taking into account the composition of the ingredients can produce organic fertilizers of a standard quality, with relatively balanced levels of NPK elements, effectively increasing the ability of biofresh biological agents, so as to increase the disease suppression index by 30.12%, with an increase in productivity of 14.31% of non-standard organic fertilizer treatments. And with the efficiency of using Biofresh biological agents of 70.6%.

Key words: Standardized organic fertilizer, The increased role of biofresh biological agents.

INTRODUCTION

The use of organic matter *that* is made into standardized organic fertilizers is very crucial to increase soil fertility, which is increasingly degraded due to the intensive use of inorganic fertilizers on plant cultivation lands. Organic fertilizers are useful as providers of macro and micro nutrients increase cation exchange capacity; can form complex compounds with metal ions that poison plants such as Al, Fe and Mn; a source of energy for microbial life that converts organic compounds into inorganic compounds and produces organic acids which promote the release of nutrients for plants. In soils with low organic matter content, the number of microbes in the soil is also low. Some of the roles of organic fertilizers that can improve the physical, chemical and biological properties of soil are the advantages of inorganic fertilizers which only provide a part of macronutrients and the longer their use, the more they reduce the fertility and quality of the soil on plant cultivation lands (Barbarick, 2006; Las *et al.* 2010).

One of the most important aspects of total nutrient balance is the C/N ratio. If the C/N ratio is high, the biological activity of the microorganisms will be reduced and if it is too low, the excess nitrogen which is not used by the microorganisms cannot be assimilated. N mineralization is influenced by the C/N ratio. Organic matter which has a low C/N ratio result in a higher mineralization rate than organic material with a high C/N ratio. A C/N ratio that is too low can also inhibit the absorption of other nutrients so that it can inhibit plant growth. A low C value can be assumed that the soil has low microorganism activity (Sholihah *et al.* 2012;

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Abera *et al.* 2012). Microbes can produce secondary metabolic compounds such as antibiotics, siderophores, *hydrogen cyanide* (HCN), produce various hydrolytic enzymes such as chitinase, protease and cellulase, fix N, dissolve phosphate and potassium and produce IAA (Indole-3-Acetic Acid), induce systemic resistance, thus play a role as a biofertilizer and biopesticide (Khaeruni *et al.* 2010; Sutariati *et al.* 2013).

The balance of the combination of N, P and K fertilizers affects the efficiency of nutrient use (Syafruddin *et al.* 2006). Excess N or K fertilization in maize plants causes vegetative growth to be more dominant than generative growth, resulting in decreased yields of maize (Grzebisz *et al.* 2014). Therefore, balanced N, P and K fertilization are needed. The provision of fertilizer in sufficient and balanced amounts is a key factor in increasing maize productivity and production (Attanandana *et al.* 2003). Availability of nutrients, structure, good soil air and light conditioning greatly affects the growth and development as well as the ability of plant roots to absorb nutrients (Nuraida *et al.* 2020). The ability of the soil to provide N is largely determined by the condition and amount of soil organic matter, the chemical properties of the soil which affect the mineralization of N in the soil which is important for increasing plant productivity (Eche *et al.* 2013).

Optimal plant growth and productivity are strongly influenced by the availability of nutrients in insufficient and balanced quantities, especially NPK levels, C-Organic content and C/N ratio. The use of organic fertilizers both in substituting a source of nutrients from inorganic fertilizers as well as the main source of nutrients for plants has not provided optimal results, this is strongly suspected because so far in making organic fertilizers it only refers to efforts to decompose organic materials without taking into account the composition, levels. and its nutritional balance. And on the other hand, various types of organic materials have varied nutritional compositions and levels and their presence is not always available at any time, place and insufficient quantities so that the organic fertilizers produced do not have clear quality standards and are relatively stable with relatively high levels of NPK elements. balanced. The purpose of this study was to analyze and formulate standardized organic fertilizers according to the Indonesian National Standard (INS 7763-2018) and the Ministry of Agriculture Regulation (MAR 70-2011) with relatively balanced levels of NPK elements and their effectiveness in enhancing the role of biological agents to increase disease resistance and maize productivity on marginal lands.

MATERIALS AND METHODS

The materials used were a cow and chicken manure, rice straw, soybean litter, rice husks, bran, procal, decomposer, molasses, clean water, Biofresh biological agents (*Bacillus subtilis ST21e, B. cereus* ST21b and *Serratia sp.* SS29a), inorganic fertilizers (Urea, SP36 and KCL), BISI-2 corn seed varieties. This research was conducted in the period of June to November 2019 at South Konawe Regency Southeast Sulawesi Indonesia and in the Integrated Laboratory of Plant Protection Department, Faculty of Agriculture, Kendari Halu Oleo University. The field research was arranged in a randomized block design consisting of 3 treatments including B_0 = Inorganic fertilizer (control); B_1 = organic fertilizer formula A + agens hayati Biofresh; B_2 = organic fertilizer formula B + agens hayati Biofresh. Each treatment was repeated 3 times, which in total there were 9 experimental units.

Making organic fertilizer formula A without considering the composition of the ingredients and the making of formula B organic fertilizer is carried out by taking into account the composition of the material which includes; C/N ratio of the total material, levels of N (Nitrogen), P (Phosphate) and K (Potassium), C-Organic and water content and pH. Therefore, the results fulfilled the quality standards of organic fertilizers in accordance with the Indonesian National Standard (INS 7763-2018) and the Ministry of Agriculture Regulation (MAR No. 70-2011). The total nutrient content of the standard organic fertilizer was calculated using equation (1) and the total ingredient ratio of C/N by using equation (2), which the authors formulated as follows:

$$Kn_y = \sum \frac{(Ax.\ Bx.\ Ny)}{(Ax.\ Bx)} \quad \text{......... (Eq 1)}$$

With; x = 1, 2, 3, ...; y = 1, 2, 3 ....

Where,

Kn= Total nutritional content of organic matter.

A= Weight of organic matter.

B= Dry weight of organic matter (%).

N= Organic nutrient content.

x= Type of organic material.

y= Organic nutrient type.

$$Tb = \sum \frac{(Ax.\ Bx.\ Cx)}{(Ax.\ Bx)} \times R \quad \text{...... (Eq 2)}$$

With; x = 1, 2, 3, ...

Where,

Tb= Total weight of material.

A= Weight of organic matter.

B= Dry weight of organic matter (%).

C= Organic C/N ratio.

x= Type of organic material.

R= Optimal C/N ratio of total organic matter.

The process of making organic fertilizer was carried out using the following procedures: Large organic materials were chopped up to ± 2 cm in size, then all ingredients were mixed and watered evenly with a decomposer solution + molasses + water and stirred until a mixture was formed, with a moisture content of ± 30%. The dough formed was made into mounds as high as ± 20 cm and tightly closed with a tarpaulin for ± 14 days (the dough was maintained at ± 40^{o}C). The finished organic fertilizer (covered with white fungus, odourless with a normal dough temperature) was dried with the wind until it reached the moisture content of ± 17%. A sampling of organics was carried out at 10 randomly, then mixed evenly (compiled) then packaged and labeled. Organic fertilizer samples were sent to the bogor Agricultural Institute Soil Science and Land Resources Laboratory for analysis.

Soil processing was carried out 2 times, then 9 beds/ treatment plots were made according to the study design, with a size of 20 m 50 m. Calcification using procal with a dose of 7 kg/beds (equivalent to 70 kg/ha). Corn planting is done 1 week after the application of organic fertilizer, employing planting holes as deep as ± 5 cm, as much as 1 seed/hole, with a spacing of 25 cm × 80 cm. The application of organic fertilizer (treatments B_1 and B_2) is carried out the day before planting by way of displacement on a row of plant lines at a dose of 500 kg/plots (equivalent to 5 tons/ha).

Application of inorganic fertilizer three times with a dose of 156 kg N/ha + 75 kg P_2O_5/ha + 110 kg K_2O/ha (Handrid *et al.* 2017). Time and dosage for each treatment (B_0 = 100%; B_1 and B_2 = 50%). Application I shortly after planting using urea fertilizer (46% N) = 11.1 kg/plots + SP36 (36% P_2O_5) = 20.8 kg/plots + KCL (60% K_2O) = 18.3 kg/plots. The second application at the age of 30 days after planting (DAP) uses urea fertilizer = 13.9 kg/plots by planting hole around the corn tree. The third application at the age of 50 DAP uses urea fertilizer = 8.9 kg/plots by planting hole around the corn tree.

The sheath blight (*Rhizoctonia solani*) disease severity was determined using 10 plants samples. Each treatment was repeated at the age of 2, 4, 6, 8 and 10 weeks after planting (WAP) was calculated using equation (3). After determining the disease severity, the AUDPC (Area Under the Disease Progress Curve) was calculated to determine the overall disease development (Paraschivu and Cotuna, 2013), by using equation (4). Furthermore, the Disease Pressure Index (DSI) was calculated to determine the effectiveness of a biocontrol agent against pathogens (Nawangsih and Wardani, 2014), by using equation (5).

$$I = \frac{\Sigma(ni.vi)}{NZ} \times 100 \qquad \text{......... (Eq 3)}$$

Where,
I= Attack Intensity (%).
ni= Number of samples with damage scale vi.
vi= Scale damage value for example i.
N= Number of plant samples observed.
Z= Highest damage scale value.
With a scale value: 0 = no attack; 1 = 1st (lowest) frond damage ≤ 25%; 3 = 1st, 2nd, 3rd frond damage > 25-50%; 5 = 1st, 2nd, 3rd frond damage > 50-75%; 7 = 1st, 2nd, 3rd frond damage > 75-90%; 9 = 1st, 2nd, 3rd frond damage > 90-100%.

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{X1 + X2}{2}\right)(t2 - ti) \qquad \text{....(Eq 4)}$$

Where,
Yi + 1 = i + 1 observation data.
Yi = i observation data.
ti + 1 = i + 1 observation time.
ti= i observation time.
n= Total number of observations.

$$DSI = \frac{Dic - Dib}{Dic} \times 100 \qquad \text{...... (Eq 5)}$$

Where,
Dic = AUDPC on the control.
Dib = AUDPC at treatment.

Analysis of salicylic acid level and the peroxide enzymes activity were carried out to determine the effect of organic fertilizer plus and the Biofresh biological agents ability as biotic elicitors in stimulating plants against diseases. Adult leaf sampling was taken in the vegetative (4 WAP) and the generative phase (8 WAP). Furthermore, the sample was extracted and analyzed at the Integrated Laboratory, Plant Protection Department, Faculty of Agriculture, Halu Oleo University Kendari. The salicylic acid level analysis was carried out using the UV-Vis spectrophotometer method (Warrier *et al.* 2013). 9 ml of the mixture contained 1 ml of leaf extract (at 1:10 g/v) and 8 ml of Fe Cl_3. 1 g of leaf sample, cut into small pieces was placed into the Erlenmeyer, then 10 ml of 10% ethanol solution was added and shook for 15 mins at a speed of 150 rpm and filtered. The filtrate was placed into the eppendorf and centrifuged for 10 mins at a speed of 12,000 rpm and 25°C, then 1% $FeCl_3$ solution was added. The absorbance level was measured at a wavelength of 525 nm.

Analysis of peroxidase enzyme activity (AOAC, 2005), was carried out by weighing 90 mg of BSA (Bovin Serum Albumin), then dissolved in 25 ml of distilled water and added with 1% NaOH and aquads, in order to obtain a standard protein solution of 3600 ppm. The standard solution was pipetted at 0.2, 0.4, 0.6, 0.8 and 1 ml respectively into a test tube and diluted with distilled water up to 6 ml. Subsequently, 6 ml of biuret reagent was added to each tubeand left for 30 mins at room temperature. Blanks mixture containing 6 ml of water and 6 ml of biuret reagent were used with 2 g of protein samples were dissolved in 20 ml of distilled water, then centrifuged for 30 mins and cooled for ± 20 mins. 1 ml of the sample filtrate was pipetted and added to Blanks mixture and left for ± 30 mins. The sample protein content was then calculated using equation (6).

$$PC\ (\%) = \frac{a}{b} \times 100 \qquad \text{......(Eq 6)}$$

Where,
PC= Protein content.
a= Weight of protein.
b= Sample weight.

The observations from the harvest yields included, the number of cobs/clumps shortly after harvesting, before and after the corn kernels were peeled off and dried using an oven for ± 15 hours at 70°C until the water content reaches ± 17%. While other observations included, the seed weight/ cob (g), Weight of 1000 seeds (g). The production of ton ha^{-1} using equation (7) was formulated as follows:

$$P = Bx\left[\frac{y\ (cm^2)}{n\ (cm^2)}\right] \qquad \text{...............(Eq 7)}$$

Where,
B= Weight of seeds/clumps.
y= Volume/ha (cm^2).
n= Volume of spacing (cm^2).

The data collected were analyzed using variance analysis. The results showed significant differences with the Duncan Multiple Range Test (DMRT) at 95% and were also used in determining the best treatment from others.

RESULTS AND DISCUSSION

Quality of organic fertilizer

The results of organic fertilizer analysis (Table 1), showed that the total elements of NPK (N 0.84% + P_2O_5 2.76% + K_2O 1.88% = 5.48%) was relatively higher than the INS standards = 2 and the MAR standards = 4 They also had a balanced, pH value of 7.7, a C-Organic level = 19% and a C/N ratio of 22.6. All the criteria analyzed in this study were used in assessing the organic fertilizers' quality.

The results of the analysis of organic fertilizers indicate that organic fertilizers which are formulated by taking into account their composition can produce organic fertilizers whose quality is in accordance with INS 7763-2018 and MAR No. 70-201. Standardized organic fertilizer (formula B) is expected to increase the ability and role of biological agents as well as the ability and independent role of organic fertilizers to increase disease resistance and productivity of maize plants on marginal lands. Standardized organic fertilizers which are expected to be suitable as a medium for growth and development of the biological agents *Bacillus subtilis*, *B. cereus* and *Serratia* sp. (Biofresh active ingredients), because biological agents will only be able to live and develop and increase their ability if the substrate as a source of nutrition and energy is available and the environmental conditions are suitable.

Biological agents that act as biopesticides are able to produce secondary metabolic compounds such as antibiotics, siderophores, *hydrogen cyanide* (HCN) and various hydrolytic enzymes such as chitinases, proteases and cellulases, production of *anti-fungal metabolites* (AFMs) and induce plant resistance and biological agents that play a role. as a biofertilizer capable of fixing N, dissolving phosphate and potassium, chelating iron with high affinity, a mechanism of competition for space and nutrients, producing auxin, as a Plant Growth Promoting Rhizobacteria (PGPR) (Kuan *et al.* 2016; Bhattacharyya *et al.* 2012; Khaeruni *et al.* 2010; Sutariati *et al.* 2013).

Plant resistance towards disease

The results of the calculation and analysis of the salicylic acid levels (SAL) and the peroxidase enzyme activity (PEA) and the percentage increase (Table 2), shows the effectiveness of standardized organic fertilizer treatment with formula B + Biofresh (B_2) capable of producing salicylic acid levels and peroxidase enzyme activity of plant tissue which tends to be higher than the treatment of organic fertilizer formula A (B_1) and control (B_0). At plant age 4 WAP was able to produce salicylic acid levels of 0.35% with a percentage increase of 775% from the control treatment and was able to produce peroxidase enzyme activity of 0.39% with a percentage increase of 117% from the control treatment. At plant age 8 WAP was able to produce salicylic acid levels of 1.17% with a percentage increase of 1.200% from the control treatment and was able to produce

Table 1: Showed the analysis results of organic fertilizers' quality, the Indonesian National Standard (INS 7763-2018) and the Ministry of Agriculture Regulation (MAR No. 70-2011).

Analyzed elements	Unit	Formula organic fertilizers*		Standard	
		A	B	INS 7763-2018**	MAR No. 70-2011***
N-Total	%	0.67	0.84	Total NPK	Total NPK
P_2O_5-Total	%	1.13	2.76		
K_2O	%	3.51	1.88		
Total NPK	%	5.31	5.48	Min. 2	Min. 4
Ca	%	1.2	1.08	Max. 25,5	
Mg	%	0.42	0.32	Max. 0,6	Max. 0,6
S.	%	0.29	0.29	-	-
Fe-total	ppm	6684	6358	Max. 15.000	Max. 9.000
Zn	ppm	145	148	Max. 5.000	Max. 5.000
C-Organic	%	18.98	19	Min. 15	Min. 15
C/N#	Ratio	28.3	22.6	15-25	15-25
pH		5.4	7.7	4-9	4-9
Water content	%	21.4	19.7	8-25	8-20
Particle size#	mm	6	2.5	2-4,75	2-5
Other ingredients#	%	0.6	0.5	Max. 2	Max. 2

Source: Laboratory test results from the department of Soil Science and Land Resources, Faculty of Agriculture Bogor Agricultural University (2018)*; National Standardization Agency (2018)**; Indonesia Ministry of Agriculture (2011)***. (Note: #= Calculation and manual analysis).

peroxidase enzyme activity of 1.08% with a percentage increase of 312% from the control treatment. The increase in salicylic acid levels from the age of 4 WAP plants to 8 WAP was 234% and peroxidase enzyme activity was 177%. These results indicate the ability of standardized organic fertilizers to increase the role of biological agents so as to increase the Disease Suppression Index (DSI).

The most important known role is as a signal in the activation of the defense response against pathogens. Salicylic acid is induced after infection by pathogens or stress due to biotic and abiotic factors. Salicylic acid plays an important role in the activation of genes that control plant resistance to pathogenic infections by inducing a protein linked to pathogenesis associated with anti-pathogens. Salicylic acid is one of the main hormonal factors that determine the conditions of stressed plants. Salicylic acid is naturally found in plants and has been shown to be involved in plant defense against pathogenic infections (Vlot *et al.* 2009).

The results of the observation of disease severity and the results of the DMRT test the effect of organic fertilizer treatment on severity of disease, area under disease-progress curve (AUDPC) and disease suppression index (DSI) (Table 3), shows the standardized organic fertilizer treatment (B_2) always has the lowest disease severity at each observation time. The results of the calculation of the AUDPC and DSI of midrib blight show that the treatment using standardized organic fertilizers + biological agents Biofresh (B_2) has the lowest AUDPC value with the highest DSI value. These results indicate that the treatment using standard organic fertilizer + Biofresh biological agent (B_2) has a very significant increase in Disease Suppression Index (DSI), which is 30.12% from the non-standard organic fertilizer treatment + Biofresh biological agent (B_1) and by 51.23% of the 100% inorganic/control fertilizer (B_0) treatment. In addition, with only one application of the Biofresh biological agent with a dose of 333 kg/ha (29.4%) of the non-standardized organic fertilizer treatment dose of 1.133 kg/ha with two applications, it can be stated that standardized organic fertilizer treatment is more effective and efficient in increasing the ability of Biofresh biological agents, with the efficiency of using Biofresh biological agents of 70.6%.

Harvest yields

The results of the observation and DMRT test of the independent effect of organic fertilizer treatment on harvest yield variables (Table 4), show the Number of Cob per tree, Seed Weight per cob and production (ton ha^{-1}) in treatment B_2 is significantly different from B_1 and B_0. Treatments of B_2 and B_1 were significantly different from treatment B_0/control on all yield variables. These results indicate that the treatment using standard organic fertilizer + biological agent Biofresh (B_2) has increased the production of tons ha^{-1} which is very significant, which is 14.31% from the non-standard organic fertilizer treatment + Biofresh biological agent (B_1) and is 37.95% of the treatment 100% inorganic fertilizer/control (B_0).

Table 2: Salicylic acid levels (SAL), enzymes peroxidase activity (EPA) and the percentage increase (%).

Treatment	Levels at plant age				Increase from 4 WAP to 8 WAP		Increase from B_0 to B_1 and B_2 (%)			
	4 WAP		8 WAP				4 WAP		8 WAP	
	SAL	EPA	SAL	EPA	SAL	EPA	SAL	EPA	SAL	EPA
B_0. Control	0.04	0.18	0.09	0.26	125	46	Control			
B_1. Formula A	0.20	0.23	1.09	0.79	445	239	400	29	1111	202
B_2. Formula B	0.35	0.39	1.17	1.08	234	177	775	117	1200	312

Source: Integrated laboratory of the Plant Protection Department, Agriculture Faculty, Halu Oleo university Kendari 2018.

Table 3: The treatment effect of organic fertilizer towards severity of disease (SoD), area under disease progress curve (AUDPC) and disease suppression index (DSI).

Treatment	Severity of disease (%)*				AUDPC	DSI
	2-4 WAP	6 WAP	8 WAP	10 WAP	(%/day)**	(%)**
B_0. Control	0.00	2.22	6.3	13.33	212.6	0.00a
B_1. Formula A	0.00	1.11	3.33	8.52	121.9	39.37b
B_2. Formula B	0.00	0.74	2.59	7.04	95.9	51.23c

Source: *Observation data; **Analysis original data.

Table 4: Effect of organic fertilizer treatment on harvest yield variables.

Treatment	Harvest yield variables			
	Number of cob/tree*	Seed weight/cob (g)*	Weight of 1000 seeds (g)*	Production (ton ha^{-1})**
B_0. Control	1.47a	113.6a	234.53a	5.56a
B_1. Formula A	1.63b	123.2b	256.53b	6.71b
B_2. Formula B	1.73c	132.9c	257.33b	7.67c

Source: *Observation data; **Analysis original data (conversion).

The ability of standardized organic fertilizers to increase disease resistance and productivity of maize plants on marginal land is strongly suspected because standard organic fertilizers contain relatively higher and balanced macronutrient content, relatively higher levels of C-Organic but with ratios that meet the standards. and neutral pH, as well as the availability of micronutrient elements in relatively more suitable quantities for plants and other criteria in accordance with the standards, are supporting factors in fulfilling the availability of nutrients for plants and substrates for Biofresh biological agents, so that simultaneously able to increase resistance to disease and productivity of maize on marginal land (Khaeruni *et al.* 2020). Las *et al.* (2010) stated that microbial activity will convert organic compounds into inorganic compounds and produce organic acids which promote the release of nutrients for plants.

The secondary metabolites formed as a result of the synergy between rhizobacteria are utilized by plants in several mechanisms of growth and resistance to pathogens (Khaeruni *et al.* 2018). Verma *et al.* (2010) explained that rhizobacteria which act as PGPR provide beneficial effects, either directly or indirectly, on plants, as stimulants of growth and protection of plants from pathogens through activities in plant root areas. These activities include the provision of NPK elements, growth hormone and the production of antibiotics which can negatively affect pathogens. Ramamoorty *et al.* (2001) stated that the application of rhizobacteria to seeds will cause reactions in plant cell walls, cell walls will modify the structure and biochemistry that will be used in the synthesis of proteins and other chemicals which will later be used in plant defense mechanisms against pathogens.

CONCLUSION

Formulation of organic fertilizers taking into account the composition of the ingredients can produce organic fertilizers whose quality is in accordance with the Indonesian National Standard (INS 7763-2018) and the Ministry of Agriculture Regulation (MAR 70-2011), with relatively balanced levels of NPK elements.

Standardized organic fertilizers effectively increase the ability of biofresh biological agents, so that they can increase the disease suppression index (DSI) which is very significant, which is 30.12% from non-standard organic fertilizer + Biofresh biological agents and 51.23% from 100% treatment inorganic fertilizer. With an increase in productivity of 14.31% from non-standard organic fertilizer treatment + Biofresh biological agents and 37.95% from 100% anoganic fertilizer treatment. And with the efficiency of using Biofresh biological agents of 70.6%.

Conflict of interest: None.

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