Efficiency of Using *Trichoderma*, *Mucor* and *Aspergillus* Antimicrobial Pellets to *Rhizophora mucronata* Poir. Planting at Abandoned Shrimp Farm in Khanom District, Nakhon Si Thammarat Province, Thailand

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Abstract

Rhizophora mucronata Poir planting was non using antimicrobial pellets in spreading pathogens of genus *Fusarium*, *Polyporus* at abandoned shrimp farm area, Khanom District, Nakhon Si Thammarat Province, Thailand. The plants were short with 33% survival rate, low macronutrient, micronutrients and supplemented nutrients. Only 9 isolates of fungal biodiversity were found. When *Trichoderma* antimicrobial pellets were used to induce 2-3-folds of growth, 95 % of *R. mucronata* plants survived. This aimed to compare the height growth, survival rate, physical and biological properties before and after using the *Trichoderma*, *Mucor* and *Aspergillus* multi-antimicrobial pellets. However, using the *Trichoderma*, *Mucor* and *Aspergillus* multi-antimicrobial pellets for *R. mucronata* planting in spreading pathogens of *Fusarium*, *Polyporus* area, the highest growth was 4.30 folds and 99% of the plants survived. Furthermore, using these pellets in non pathogens, the growth was 4.02 folds and 99% of the plants survived, while the growth was 2.64 folds and 88% of the plants survived in the spreading area of these pellets. On the other hand, in the controlled area the growth was 0.42 folds and 88% of the plants survived. When the *Trichoderma, Mucor* and *Aspergillus* multi-antimicrobial secreted 1-aminocyclopropane-1-carboxylate (ACC) extraenzymes, ethylene synthesis decreased. When this ethylene in the plant reduced, the antimicrobial secreted. This induced IAA hormone to speed up the plants growth, dissolved phosphorus and fixed nitrogen. Macronutrients, micronutrients and supplemented nutrients increased in sediment soil between 45 to 2000%, while heavy metals reduced between 80 to 100%. The coefficients were more accurate than 95.5. So government should develop *R. mucronata* or other mangrove plants in spread or non spread pathogens area by using *Trichoderma, Mucor* and *Aspergillus* multi-antimicrobial pellets in abandoned shrimp farm. Consequently, the area will be restored and returned to natural balance within 5 years.

Keywords: *Trichoderma,* multi-Antimicrobial Pellets*,* *Rhizophora mucronata*, Survival, Growth, Mangrove forest

Introduction

In 2012 we surveyed abadoned shrimp farm area spreading pathogens. in Khanom district, Nakhon Si Thammarat province. The pathogens were *Fussarium, Polyporus.* (Sakayaroj et al., 2012, Preedanon et al., 2017) The area contained less nutrients such as nitrogen (N), phosphorus (P) potassium (K) calcium (Ca), magnesium (Mg), contamination of lead(Pb), cadmium(Cd), mercury (Hg) and only 9 isolations fungal biodiversity existed. (Rattanaloeadnusorn, Thitaya, Sujaya, & Sirikhae, 2012) The growth of *Rhizophora. mucronata*, *Rhizophora apiculata* etc. were high while survival rate and density were less than 33%, and mortality increased. Figure 1 (Rattanaloeadnusorn et al., 2014) After the *Trichoderma* antimicrobial pellets were used in the restoration of the mangrove forest areas at abandoned shrimp farm area, sediment soil at Khok Kham District, Samut Sakhon Province, Thailand, it was found that *R. mucronata* plants were high, leaves width and girth at breast height (GBH) were grater than nornal 2- 3 folds and restored to the natural balance within 5 years, (Rattanaloeadnusorn et al., 2014 Bryant, Nielsen, & Tangley, 1997) while it took 10-15 years without using

Trichoderma antimicrobial pellets. (Ahemad & Malik, 2011 Aksornkoae & Khemnark, 1994 Wijarn, 2009) Later multi- antimicrobial pellets were produced by bio- technology coperated with industry, IFOAM standard. Their distinctive features were 1.) To induce the growth of leaves, stems, roots. as a result of Bryant et al., 1997; Glick, Cheng, Czarny, Cheng, and Duan, 2007; Lavakush, Janardan, Jay, Jaiswal, and Kumar, 2014; Rattanaloeadnusorn, 2017. 2.) To speed up the decomposition of organic and inorganic substances that other microorganisms cannot do in order to induced plants growth. as a result of Glick et al., 2007; Ahemad and Malik, 2011; Rattanaloeadnusorn, 2017. 3.) To control pathogens as a result of Lavakush et al., 2014; Ahemad and Malik, 2011; Rattanaloeadnusorn, 2017. 4.) To remove heavy metals from soil and adjust its pH. as a result of Vaxevanidou, 2008; Harris & Sommers, 1968; Rattanaloeadnusorn, 2017. Figure2

From the above reasons, we studied the height growth, survival rate, nutrients inducing of *R. mucronata* planting with *Trichoderma, Mucor* and *Aspergillus* multi-antimicrobial pellets that the antimicrobial were isolated from the soil mangrove forest in abandoned shrimp farm area in Khanom District, Nakhon Si Thammarat Province, Thailand. This aimed to study the efficiency of the *Trichoderma*, *Mucor* and *Aspergillus* multi-antimicrobial pellets for *R. mucronata* plants in 4 areas of Khanom District, Nakhon Si Thammarat province, Thailand: 1.) spreading pathogens with these pellets 2.) non spreading pathogens with these pellets 3.) spreading pathogens without using these pellets 4.) controlled area in abandoned shrimp farm area.

Figure1 Disease on *Xylocarpus granatum* and *Rhizophola apiculata* at Khanom District, Nakhon Si Thammarat Province, Thailand.

Figure 2 A.) Innovation of *Trichoderma pellets* B.) *Trichoderma*, *Mucor, Aspergillus* multi- antimicrobial pellets C.) THAN multi-antimicrobial, IFOAM standard for the restoration of *R. mucronata* plant at spread pathogens in soil, low nutrients and heavy metal contamination.

Materials and Methods

Area Studies

Khanom District in Nakhon Si Thammarat Province, Thailand. This aimed to study the efficiency of the *Trichoderma*, *Mucor* and *Aspergillus* multi-antimicrobial pellets, IFOAM standard for *R. mucronata* plants in 4 areas of Khanom District, Nakhon Si Thammarat province, Thailand: 1.) spreading pathogens with these pellets 2.) non spreading pathogens with these pellets 3.) spreading pathogens without using these pellets 4.) controlled area in abandoned shrimp farm area. Table 1

Table 1 Design of experiment of *Trichoderma*, *Mucor* and *Aspergillus* multi-antimicrobial pellets, IFOAM standard for the restoration of *R. mucronata* plant at spread pathogens in soil, low nutrients and heavy metal contamination.

Area1		**Area2**		**Area3**		**Area4**	
pathogen	Multi-antimicrobial	pathogen	Multi-antimicrobial	pathogen	Multi-antimicrobial	pathogen	Multi-antimicrobial
yes	yes	no	yes	yes	no	no	no

Materials and Methods

Randomized Completes Design (RCD) was used to treat 4 areas: 1.) spreading pathogens with these pellets 2.) non spreading pathogens with these pellets 3.) spreading pathogens without using these pellets 4.) controlled area in abandoned shrimp farm area. 20 plants were employed. This aimed to compare the height growth, survival rate, physical and biological properties before and after using the *Trichoderma*, *Mucor* and *Aspergillus* antimicrobial pellets at abandoned shrimp farm in Khanom District, Nakhon Si Thammarat Province, Thailand. The procedures were as follows: Figure 3

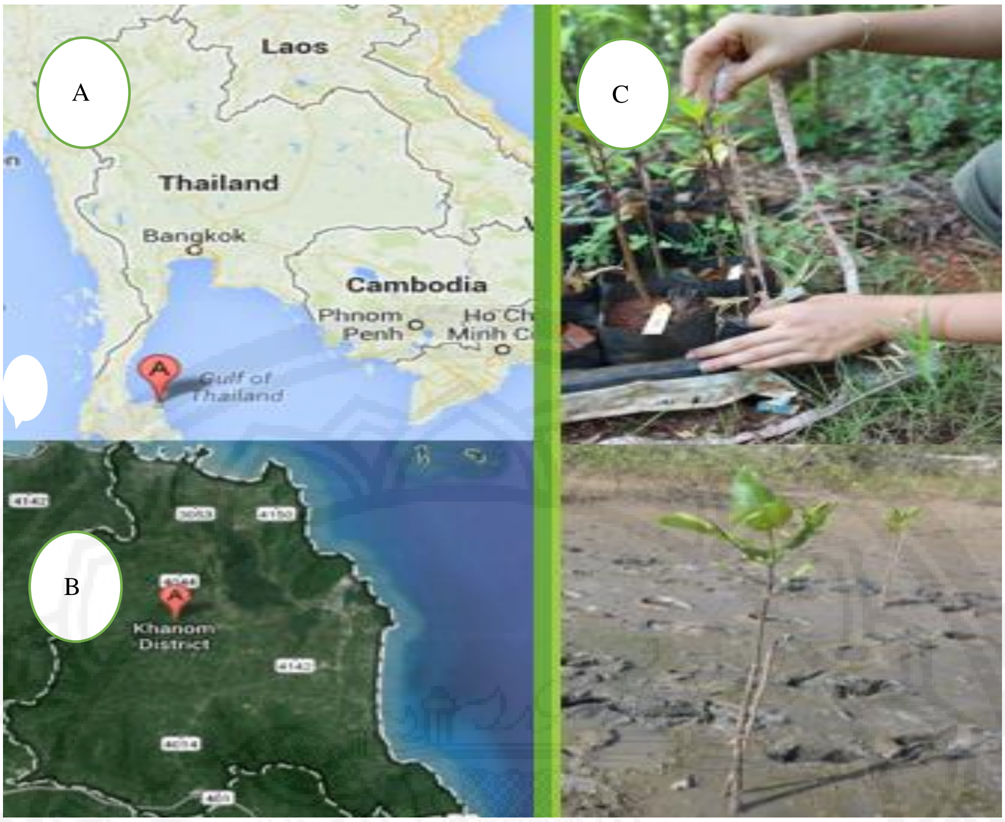


Figure 3 Areas studied, A.) Nakhon Si Thammarat Province, Thailand. B.) Khanom District, Nakhon Si Thammarat Province, Thailand. C.) seedlings and height growth and survival rate of *R. macronata* adding the *Trichoderma*, *Mucor* and *Aspergillus* multi-antimicrobial pellets.

1. Soil Sampling and measuring major and minor nutrients, heavy metals namly mercury, lead, cadmium and pH, according to the method of Vaxevanidou, 2008 Harris & Sommers, 1968.

2. Isolation and purification and preservation of pure fungal biodiversity in the soil by soil and dilution plate method according to the method of Harris & Sommers, 1968.

3. Plant *Rhizophora mucronata* seedlings age 6 months according to the method of Aksornkoae and Khemnark 1994. Added 30 grams *Trichoderma, Mucor* and *Aspergillus* multi-antimicrobial pellets.

4. Plant *R. mucronata* seedlings in spreading pathogen area in 1-foot-deep soil. Then observe and measure the height growth and its survival every 2 months.

5. Analyze the height growth and survival rate after 1 year planting *R. mucronata* plants adding the *Trichoderma, Mucor* and *Aspergillus* multi-antimicrobial pellets by Microsoft Excel Professional Plus 2013 and SPSS. Then calculate the percentage of the height growth compared to the control, coefficient of determination (R^2) and the ability to predict the percentage correctly. Lastly, write the physical and biological changes before and after planting the *R. macronata* added the *Trichoderma, Mucor* and *Aspergillus* multi-antimicrobial pellets. (Behera et al., 2017)

6. Predict *R. macronata* plant growth added the multi-antimicrobial pellets according to this eguation.

Exponential growth = lnw2-lnw1/(t2-t1) …. (1) Which is K=constant, e=constant, t=time to study, w=height

Results

The results showed that using the *Trichoderma*, *Mucor* and *Aspergillus* multi- antimicrobial pellets for *R. mucronata* planting in spreading pathogens of *Fusarium*, *Polyporus* area, the highest growth was 4.30 folds and 99% of the plants survived. Furthermore, using these pellets in non pathogens, the growth was 4.02 folds and 99% of the plants survived, while the growth was 2.64 folds and 88% of the plants survived in the spreading area of these pellets. On the other hand, in the controlled area the growth was 0.42 folds and 88% of the plants survived. Figure 4 - 6 Table 2 After 1 year of planting *R. macronata plants* added *Trichoderma, Mucor* and *Aspergillus* multi- antimicrobial pellets, macronutrients, micronutrients and supplemented nutrients increased in sediment soil between 45 to 2,000%, while heavy metals reduced between 80 to 100%. Furthermore, the fungi biodiversity increased from 9 islations to 21 isolations. Table 3 (Rattanaloeadnusorn et al. , 2012 Rattanaloeadnusorn et al., 2014) When using only *Trichoderma* antimicrobial pellets in non spread area of pathogens in abandoned shrimp farm in Khok Kham District, Samut Sakhon Province, Thailand, the height was 2 folds. (Rattanaloeadnusorn et al., 2014)

The equation of the exponential growth for *R. mucronata* plants added multi-antimicrobial pellets resulted in 0.99 coefficient of determination (R^2) and 95.5 percent. This equation can predict coefficient of determination and percentage accurately. Figure 5-6 The equation of the exponential growth was as follows:

Exponential growth = lnw2-lnw1/(t2-t1) …. (1) Which is K=constant, e=constant, t=time to study, w=height

The resuts of this equation of exponential growth of *R. macronata* added *Trichoderma, Mucor* and *Aspergillus* multi- antimicrobial pellets with the spread pathogen in Khanom, Nakhon Si Thammarat province showed that its natural balance can be restorted within 5 years. The coefficient of determination (R^2) was 99 and the predicted percentage was 95.5. On the other hand, restoring its natural balance of plating *R. macronata* without adding *Trichoderma, Mucor* and *Aspergillus* multi- antimicrobial pellets and non spreading the pathogens (contraolled area) took 10 years. (Rattanaloeadnusorn et al., 2012 Wijarn, 2009 Behera et al., 2017)

The highest growth and survived reasoning in area 4 was better than area1. Due to the *Trichoderma, Mucor* and *Aspergillus* multi-antimicrobial secreted 1-aminocyclopropane-1-carboxylate (ACC) extraenzymes, ethylene synthesis decreased. When this ethylene in the plant reduced, the antimicrobial secreted. This induced IAA hormone to speed up the plants growth, dissolved phosphorus and fixed nitrogen. macronutrients, micronutrients and supplemented nutrients increased in sediment soil between 45 to 2,000%, while heavy metals reduced between 80 to 100%. The coefficients were more accurate than 95.5.

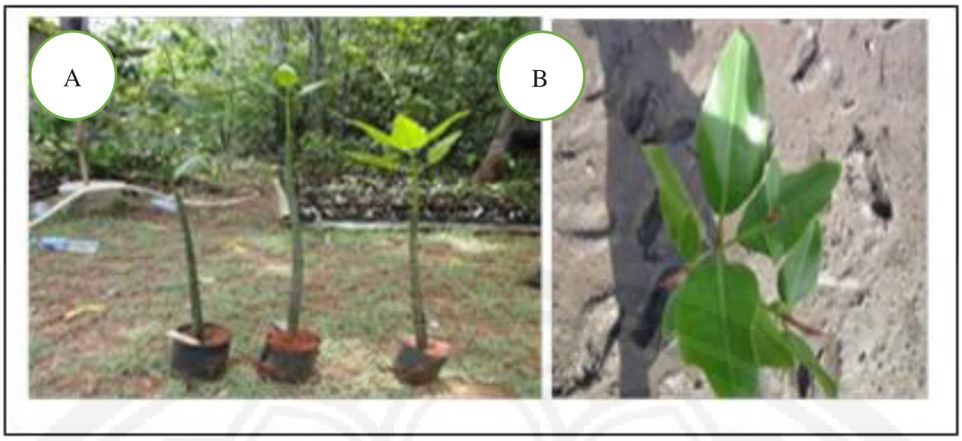


Figure 4 A) Comparison of the growth of *R. mucronata* seedlings adding multi-antimicrobial pellets (right), non- using multi-antagonistic pellets (left). B) The growth of *R. mucronata* added *Trichoderma*, *Mucor* and *Aspergillus* multi-antimicrobial pellets and the spread of the pathogen at abandons shrimp farm area in Khanom District in Nakhon Si Thammarat Province, Thailand., 6 months' age

Table 2 Height growth and plants survived of *R. mucronata* adding multi- antimicrobial pellets at abandons shrimp farm area in Khanom District in Nakhon Si Thammarat Province, Thailand., 12 months' age.

lists	Area1	Area2	Area3	Area4
Height growth (folds)	4.3	4.2	2.64	0.42
% plants survived	99	99	88	88

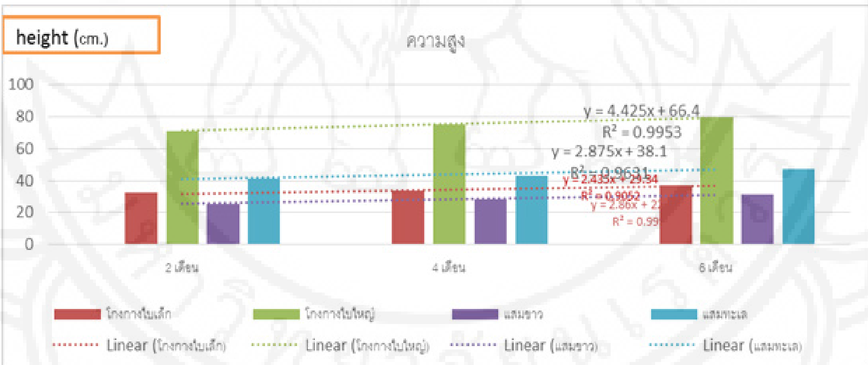
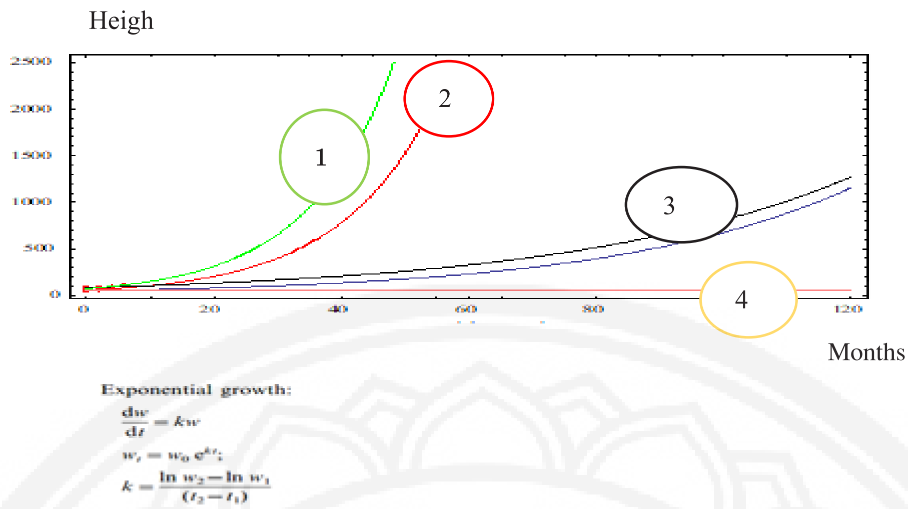


Figure 5 Analyze the height growth of *R. mucronata, R. apiculata, Avicennea alba* and *A. marina* seedlings added *Trichoderma*, *Mucor* and *Aspergillus* multi- antimicrobial pellets with a spreading pathogen every 2 months by Microsoft Excel Professional Plus 2013 and SPSS at abandoned shrimp farm area in Khanom District in Nakhon Si Thammarat Province, Thailand.



1.) spreading pathogens with these pellets 2.) non spreading pathogens with these pellets 3.) spreading pathogens without using these pellets 4.) controlled area in abandoned shrimp farm area.

Figure 6 Prototype model and predicted height growth of *R. mucronata* added *Trichoderma*, *Mucor* and *Aspergillus* multi-antimicrobial pellets in the spread pathogenic in abandoned shrimp farm and natural mangroves forest, 120 months of age, Khanom District in Nakhon Si Thammarat Province, Thailand.

Table 3 Measure the physical and biological properties before and after planting of *R. mucronata* added *Trichoderma*, *Mucor* and *Aspergillus* multi-antimicrobial pellets in the spread pathogenic in abandoned shrimp farm and natural mangroves forest.

Biological properties		Physical properties			
Number of genus fungi		% Reduced heavey		% Increaded nutrients	
before planting	after planting	before planting	after planting	before planting	after planting
9	21	-	100	45	2,000

Discussion

Rhizophora mucronata Poir planting was non using antimicrobial pellets in spreading pathogens of genus *Fusarium*, *Polyporus* at abandoned shrimp farm area, Khanom District, Nakhon Si Thammarat Province, Thailand. The plants were short with 33% survival rate, low macronutrient, micronutrients and supplemented nutrients. Only 9 isolates of fungal biodiversity were found. When only *Trichoderma* antimicrobial pellets were used to induce 2-3-folds of growth, 95 % of *R. mucronata* plants survived. However, using the *Trichoderma*, *Mucor* and *Aspergillus* multi-antimicrobial pellets for *R. mucronata* planting in spreading pathogens of *Fusarium*, *Polyporus* area, the highest growth was 4.30 folds and 99% of the plants survived. Furthermore, using these pellets in non pathogens, the growth was 4.02 folds and 99% of the plants survived, while the growth was 2.64 folds and 88% of the plants survived in the spreading area of these pellets. On the other hand, in the controlled area the growth was 0.42 folds and 88% of the plants survived.

This was caused by mycelium of the *Trichoderma Mucor* and *Aspergillus* multi- antimicrobial inserting in mycelium pathogens and secreting enzymes namely hemicellulose (cellulose), lactase (non- phenolic compounds), peroxidase(lignin). These enzymes speeded up the decomposition of organic and inorganic compounds and reduced heavy metals contamination in the soil. The efficiency of using the *Trichoderma*, *Mucor* and *Aspergillus* multi-antimicrobial pellets was better than using only *Trichoderma* antimicrobial pellets at 2.3 folds. (Glick et al., 2007; Govindasamy, Senthilkumar, Mageshwaran, & Annapurna, 2008) It can be seen that the *R. mucronata* plants with more than one isolation of antimicrobial microorganism pellets were better than the growth of only one isolation of antimicrobial microorganism. The results of this study corresponded with the study of Glick et al., 2007; Vaxevanidou, 2008; Intana, Chamswang, Intanoo, Hongprayoo, and Sivasithamparam, 2003; Sofia, 1998; Govindasamy et al., 2008. This was because antimicrobial microorganism secreted biological substances such as macro, micro and spplemented nutrients, protein, amino acid, chitosan, humic acid, fuvic acid, enzymes1-aminocyclopropane-1-carboxylate(ACC) deaminase to degrade ACC, a precursor of ethylene synthesis to induce the plant growth. This reduced the level of ethylene in the plants (Glick et al., 2007; Vaxevanidou, 2008; Figueiredo, Burity, MartI nez, & Chanway, 2008) and IAA hormone in root growth. In addition, the phosphorus dissolved and nitrogen fixed. (Lavakush et al., 2014; Ahemad & Malik., 2011) Thus, the height growth and suvival rate were higher than that of antimicrobial pellets. This can reduce 30-40 percent of fertilizer use. The plants grow very well, without any diseases and within 5 years of natural balance. (Lavakush et al., 2014 Vaxevanidou, 2008)

Conclusion and Suggestions

The study of efficiency of *Trichoderma*, *Mucor* and *Aspergillus* multi-antimicrobial pellets of *Rhizophora mucronata* Poir. planting at abandoned shrimp farm in Khanom District, Nakhon Si Thammarat Province, Thailand revealed that using the *Trichoderma*, *Mucor* and *Aspergillus* multi-antimicrobial pellets for *R. mucronata* planting in spreading pathogens of *Fusarium*, *Polyporus* area, the highest growth was 4.30 folds and 99% of the plants survived. Furthermore, using these pellets in non pathogens, the growth was 4.02 folds and 99% of the plants survived, while the growth was 2.64 folds and 88% of the plants survived in the spreading area of these pellets. On the other hand, in the controlled area the growth was 0.42 folds and 88% of the plants survived. Macronutrients, micronutrients and supplemented nutrients increased in sediment soil between 45 to 2,000%, while heavy metals reduced between 80 to 100%, and fungal biodiversity increased from 9 to 21 isoloations. When analyzing data and mathematical equations for height growth and prototyping simulation, it was found that the coefficients were 95.5. So the government should develop *R. mucronata* or other mangrove plants in spread or non spread pathogens area by using *Trichoderma, Mucor* and *Aspergillus* multi-antimicrobial pellets in abandoned shrimp farm. Consequently, the area will be restored and returned to natural balance within 5 years. Moreover, this method can induce height growth, increaed survival rate, increase fungal biodiversity, reduce the amount of heavy metals and increase the mangrove forest areas as the prototype model.

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