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Antagonism activity of phosphate solubilizing microbes and nitrogen fixing bacteria toward *Fusarium* sp.

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***Abstract*— *The ability of phosphate solubilizing microbes and N-fixing bacteria as biofertilizers to increase growth and soil P-dissolve, N soil, they also have the potential to suppress disease, The purpose of this experiment to study antagonism activity of phosphate solubilizing microbes and N-fixing bacteria to inhibit pathogen isolate. In vitro antagonism test was carried out to test the ability of isolates biofertilizers as biocontrol The isolate of phosphate solubilizing microbes were Pseudomonas mallei, Pseudomonas cepacea, Bacilkussubtilis, Bacillus megaterium, Penicilliumsp.,and Aspergillusniger while the isolate ofN-fixing bacteria were (Azotobactervinelandii, Azotobacterchroococcum and Azospirillumsp. Fusarium sp. as pathogen was used for antagonism test. The experiment showed that phosphate solubilizing microbes and N-fixing bacteria have ability to inhibit growth of Fusariumsp. as pathogen. The results of the antagonism test of the biofertilizer isolates tested by Azospirillum were able to inhibit the growth of Fusariumsp. higher than other isolates.Furthermore, from this study it can be developed that biofertilizers are not only to increase soil nutrient availability and plant growth but can be used as biocontrol.*

Keywords*— *antagonistic, biocontrol, biofertilizers, and in vitro.

I. INTRODUCTION

The continuous use of inorganic fertilizers can reduce soil quality, including reducing soil organic matter content, destroying soil structure, and reducing the population and biodiversity of soil organisms. The continuous use of synthetic pesticides can also cause negative impacts, including resistance to pathogens, the killing of beneficial non-target organisms, environmental pollution, and the presence of pesticide residues on agricultural products (Aktar et al., 2009). Therefore, it is necessary to develop environmentally friendly fertilizers and pesticides, including a consortium of growth-promoting microbes and biological agents that can function as biofertilizers and biopesticides as biocontrol.

Growth-promoting microbes that act as biological fertilizers have been developed, namely phosphate solubilizing microbe (PSM) *Pseudomonas malei*, *Penicillium* sp. and *Aspergillus Niger* which have known their ability to dissolve phosphate and the production of phosphatase enzymes and are able to produce growth regulators (Fitriatin et al., 2014 ; Fitriatin et al, 2020) . The research by Vassileva et al. (2006) reported that phosphate solubilizing microbes can produce growth regulators, siderophores and lytic enzymes to suppress the growth of pathogens. Further studies have shown the ability of PSM to synthesize metabolites to chelate metals (Vassileva et al., 2010). Research by Walpola& Yoon (2013) showed that phosphate solubilizing bacteria (*Pantoeaagglomerans* and *Burkholderiaanthina*) can produce IAA, ammonia, hydrogen cyanide (HCN), and siderophore and can increase plant growth and P uptake and resistance to disease.

Fusarium wilt control is recommended such as the use of fungicides and technical culture, but has not been able to suppress the disease (Thangavelu&Mustaffa, 2012).Biological control is directed at the development of research on antagonistic agents. Bacillus is a genus of bacteria that is reported to be able to increase plant resistance (Rebib et al. 2012). Bacillus is able to produce antifungal compounds that cause swelling of Foc hyphae in vitro (Arrebola et al. 2010)

Microbes that promote plant growth are able to control disease (Nakkeeran et al., 2005). Microbes that can be used as biological fertilizers such as *Azotobacterchroococcum* (Mali &Bodhankar, 2009) can also suppress disease. Therefore, research is needed to develop biofertilizer formulations (P-solubilizing microbes and N fixers) that act as biocontrol to increase nutrient availability and plant yields. The objective of research to study antagonism activity of phosphate solubilizing microbes and N-fixing bacteria to inhibit pathogen isolate.

II. MATERIAL AND METHODS

The antagonism test was carried out by invitro to determine the ability of isolates in biofertilizers, namely P-solubilizing microbes and N-fixers in inhibiting disease fungi. The isolates tested as biofertilizers werephosphate solubilizing microbes (*Pseudomonas mallei*, *Pseudomonas cepacea*, *Bacilkussubtilis*, *Bacillus megaterium*, *Penicillium*sp., *and Aspergillusniger)* while the isolate of N-fixing bacteria were (*Azotobactervinelandii*, *Azotobacterchroococcum*and *Azospirillum*sp. *Fusarium* sp. as pathogen isolate was used for antagonism test.

The antagonism test medium used Nutrient Agar for all isolates. The dilutions of the test isolates (P-solubilizing microbes and N fixers) were 10^{-4} and 10^{-5}). This antagonistic test uses an inhibition zone indicator or clear zone that occurs characterizing the presence of inhibition of the test isolate against *Fusarium* as a pathogen. The test isolates were inoculated by streaking / scratching on petridish with NA media after which disc paper soaked in *Fusarium* isolate was planted. The zone of inhibition that occurs was measured to see how much inhibition of P-solubilizing microbes and N fixer isolates on *Fusarium*growth (Figure 1).

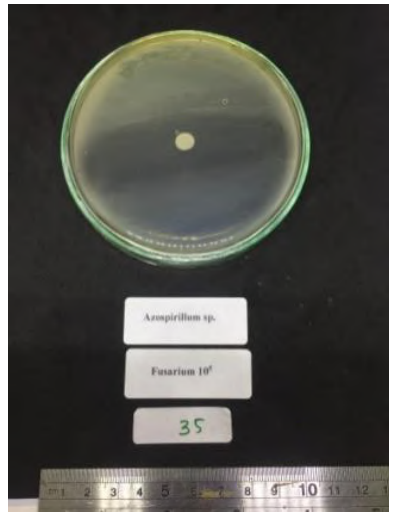

Fig.1: Antagonistic test of Azospirillumsp. isolates sp. towardFusariumsp.

III. RESULTS AND DISCUSSION

Antagonistic tests of biofertilizers (P-solubilizing microbes and N-fixer isolates against disease isolates (*Fusarium*sp.) can be found from Table 1**.**

Table 1. Antagonistic tests between biofertilizer and Fusarium sp. (3 days after inoculation/DAI)

Isolates	**Zone of inhibition(mm)**
Pseudomonas mallei	1,3
Pseudomonas cepaceae	1,65
Bacillus subtillis	1,9
Bacillus megaterium	2,1
Azotobactervinelandii	1,5
*Azospirillum*sp.	5,1
Azotobacterchroococcum	1,8
*Penicillium*sp.	1,5
Aspergillusniger	0,8

The results of the antagosnistic test on 3 DAI showed that P-solubilizing microbes (*Pseudomonas mallei*, *P. cepaceae*, *Bacillus subtillis*, *B.megaterium*, *Penicilliumsp.* and *Aspergillusniger*) and N-fixing bacteria

(*Azotobactervinelandii*, *Azotobacterchroococcum*, *Azospirillum* sp.) inhibited the growth of *Fusarium*sp. The inhibition zone or clear zone found in petridish (ranging from 0.8-5.1 mm), these isolate were able to be a biocontrol because it could be inhibit the growth of *Fusarium*sp. This was presumably because biofertilizers isolates produces secondary metabolites which can inhibit growth of *Fusarium* sp. This is supported by Raaijmakers & Mazzola (2012) that soil beneficial produced antibiotic to suppress disease.

The phosphate solubilizing fungi (*Aspergillusniger*, *Penicillium* sp.) shown their inhibition at DAI have diameter of clear zone were smaller. This is presumably because the growth of fungi is longer than bacteria,Based on the antagosnistic test showed that *Azospirillum* was able to inhibit the growth of *Fusarium* sp. higher than other isolates. Different results were shown by Widyantoro et al (2020) that *Azospirillum* had not been able to prevent the plant wilt.

IV. CONCLUSIONS

Base on in vitro antagonism test showed that biofertiizers (phosphate solubilizing microbes and N-fixing bacteria) have ability to inhibit growth of *Fusarium*sp. as pathogen. The results of the experiment were found that *Azospirillum* were able to inhibit the growth of *Fusarium*sp. higher than other isolates. Furthermore, from this study it can be developed that biofertilizers are not only to increase soil nutrient availability and plant growth but can be used as biocontrol.

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