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SOIL FUNGAL DIVERSITY IN CABBAGE HABITATS WITH AND WITHOUT CLUBROOT SYMPTOM

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Abstract:-

Cabbage (Brassica oleracea L.) was a vegetable crops cultivated in the highlands to meet the needs of the community vegetable. The main obstacle was the cultivation of cabbage root disease outbreak mace (clubroot), which until now have not found an effective control techniques. Clubroot disease caused by organisms that resemble fungi: Plasmodiophora brassicae Wor. Which was the soil inhibitant and soil borne pathogen. Therefore, there must be a way to control environmentally friendly by using suppressive soil, find microbes antagonists related to the cabbage plant habitat in the soil. The results showed that the index of diversity both on suppressive and conducive soil of 1.2304 and 1.2811 respectively, and the index of dominance on the suppressive and conducive soil were 0.6677 and 0.6838. Prevalence micoflora of the suppressive soil amounted to 44.22 % and 43.06 % conducive soil all dominated by Fusarium spp. Microbial antagonist as a potential control of P. brassicae was Trichoderma sp . Based on the analysis in the suppressive soil as much as 171 x 10^3 cfu /g soil, higher than on the conducive soil to 90 x 10^3 cfu /g soil.

Keywords: *Cabbage (Brassica oleracea L.), supperessive and condussive soil, Palmodiophora brassicae, prevalence, and soil inhibitant.*

BACKGROUND

Cabbage (*Brassica oleracea* L.) has long been cultivated as a crop of vegetables and a source of vitamins, minerals and fiber (Keinath *et al.*, 2006). In Bali cabbage plants cultivated in the highlands as Baturiti Tabanan, Candikuning-Buleleng and Kintamani Bangli. According Semangun (1989) cabbage plants are grown today have suffered Clubroot caused by the fungus *Plasmodiophora brassicae* Wor.

Losses caused by clubroot disease on cabbage plants in Britain, Germany, the USA, Asia and South Africa reach 50-100%. In Australia these pathogens cause yield losses of about 10% every year with a revenue loss of US $ 13 million. In Indonesia, the disease causes damage to the cabbage around 88.60% and in plants caisin approximately 5.42 to 64.81 % (Cicu, 2006) .

Root disease control efforts to clubroot disease need alternative ways that are environmentally friendly, efficient and effective than just relying on synthetic pesticides. For the use of suppressive soil with the role of microbes in it are expected to be more promising for the control of this disease (Garbeva *et al*., 2004). Microbes in the soil number and type very much, depending on soil fertility. Conklin (2008) states in a single gram of soil there are 108-109 kinds of bacteria. actinomycetes 107-108 per gram of soil type, there are 105-106 fungal propagules per gram of soil. These microbes (bacteria, actinomycetes and fungi) large enough in the soil. Kalia and Gupta (2005) states that the conservation measures necessary if already know the usefulness of such microbial diversity: role in the biogeochemical cycles of nutrients, sustainable land use, the microbial products for agriculture, biodegradation of xenobiotics and utilization for the industry.

MATERIALS AND MATHODS

Place and Time Research

The research was conducted at two sites in the field , namely the center of production of cabbage Bali in Candi Kuning-Tabanan, and Panca Sari, Buleleng. The study was conducted from March to June 2015. After the sample was obtained followed by analysis of soil microbes in laboratory of Plant Pathology Faculty of Agriculture, University of Udayana .

Research in the Field

Soil samples were obtained from the plant rhizosphere habitat sick and healthy cabbage taken in the area of the cabbage plant centers in Bali. Cabbage plants are ill observed, the number of clumps, the number of diseased plants, so that the disease can be determined by using the formula: number of diseased plants divided by the whole plant were observed times 100%. Data retrieval land was taken by the survey results in healthy and diseased plant centers by taking soil near the roots of cabbage plants healthy and sick with approximately 20 cm into each of 100 grams, each plant is taken 4 times then mixed evenly.

Research in Laboratory

a. Population determines Mikoflora Land

Soil samples of one gram dissolved in sterile water then mixed and divortek . Soil suspension diluted to reach a volume of 10 ml . Dilution terraced 10^{-2} - 10^{-7} under sterile conditions. The suspension was taken 1 ml is poured into a Petri dish with culture medium. Media potato dextrose agar (PDA) with a mixture of potatoes 200g, 15g sugar, to 20g in 1000 ml of distilled water and livoplosaxin (antibacterial antibiotics) with a concentration of 0.1% (w/v) were used for the isolation of fungi. Five Petri dish prepared for each solution. Cultures were incubated in the dark at a temperature of 27 ± 2°C, then colonies counted as colony forming units (CFU). A single colony was transferred into a new Petri dish containing PDA medium and incubated at room temperature . Isolates were identified macroscopically after 3 days old to determine the colony color and growth rates, and the identification of microscopically to determine septa in hyphae, form spores/conidia and sporangiophore (Samson *et al*., 1981; Pitt and Hocking, 1997; Barnett and Hunter, 1998; Indrawati *et al*., 1999).

b . The index determines the Soil Microbial Diversity and Dominance

Soil microbial diversity and dominance can be determined by calculating the ShannonWiener diversity index (Odum, 1971) and the dominance of soil microbes are calculated by counting the Simpson index (Pirzan and Pong- Cook, 2008).

(1) Index microbial diversity

The index is determined by soil microbial diversity index Shannon-Wiener diversity is by formula (Odum, 1971):

$$H' = - \sum_{i=1}^{s} Pi \ ln \ Pi.$$

Where:

H ' = Shannon - Wiener diversity index

S = Number genus

Pi = ni/N as a proportion of all i ni = total number of individual types of microbes total i, N = total number of individuals in total n)

The criteria used to interpret the diversity of Shannon-Wiener (Ferianita-Fachrul *et al*., 2005), namely: H'nilainya < 1, meaning low diversity, H ' value 1-3 means diversity classified as moderate and H ' values > 3 means diversity classified high.

(2) Index domination

Soil microbial dominance index is calculated by counting Simpson index (Pirzan and Pong- Cook, 2008), with the following formula:

$$C = \sum_{i=1}^{S} Pi^2$$

Where:

C = index Simpson

S = Number genus

Pi = ni/N ie, the proportion of an individual species i and all individuals (ni = total number of individual types i, N = total number of individuals in total n)

Furthermore, species dominance index (D) can be calculated by formula 1- C (Rad *et al*. 2009). The criteria used to interpret the dominant species of soil microbes namely: approaching 0 = index low or lower dominance by a microbial species or not there is a species which is extreme dominate other species, approaching 1 = index greater or tends domination by several species of microbes (Pirzan and Pong-Cook, 2008).

(3) Chi kuadrat analysis

Soil microbial diversity in habitat cabbage plants with and without symptoms of illness more or less can be proved by using Chi square analysis (X^2), or t test with the formula (Gomes and Gomes, 2007; Sugiyono, 2009):

$$X^2 = \sum_{i=1}^{p} \frac{(n_i - E_i)^2}{E}$$

Where:

p = number of class ni = number of units was observed that belongs to the class

i Ei = the number of units that are expected to belong to a class i

Furthermore Chi squared value arithmetic compared with Chi squared table, when Chi squared count is smaller than the table, then Ho is accepted and vice versa.

RESULTS AND DISCUSSION

Soil Fungus Population

The population of soil fungi in three locations either on the ground or on the ground conducive suppressive shows a highly significant difference. At the location of the first number of colonies that can be found in soil suppressive much as 22 x 10^3 cfu/g soil, the soil is conducive much as 10 x 10^3 cfu/g soil, on the location of the second ground suppressive much as 90 x 10^3 cfu/g soil and soil conducive 49 x 103 cfu/g soil, on the location of the third on a suppressive soil as much as 75 x 10^3 cfu/g soil, and the soil is conducive 40 x 10^3 cfu/g soil (Figure 1).

This shows that on the ground there are many micoflora suppressive soil than in soil conducive. Means one of there as microbial antagonist that suppresses the development of disease-causing pathogens root mace in three locations. Land for the plant pathogen is a hospital, either through restrictions on the length of survival or growth of pathogens.

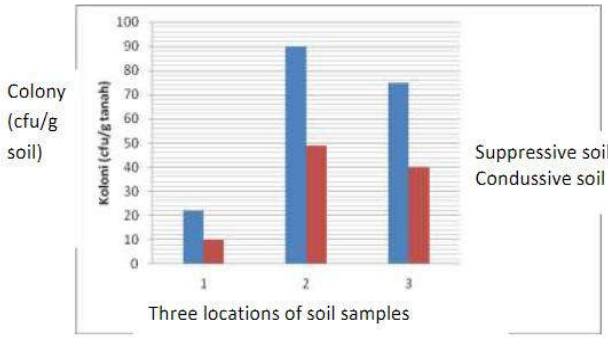

Figure 1. Colony of micoflora in the suppressive and condussive soil

Each land known to suppress pathogens or diseases. Suppressive soil is determined by soil microbial diversity, while soil microbial diversity is influenced by three main things : (a) the type of crop that is a major determinant of the structure of microbial communities in the soil, such as plant a major provider of carbon and energy sources, (b) the type of soil is the main determinant of microbial communities, just as the combination of the structure and soil texture, organic matter, stability microagregat, pH, and the presence of nutrients keys such as N, P, and Fe determine habitat in the soil, and (c) how to manage the agricultural crop rotation, soil processing, herbicides, fertilizer application and irrigation also determine the soil microbial community structure (Nannipieri, *et al*., 2003; Garbeva *et al*., 2004).

Based on observations during the study found only seven species of fungi that colonize the suppressive soil habitat and soil conducive. Seventh fungi include: Aspergillus spp., *Aspergillus flavus*, *Aspergillus niger, Fusarium* spp., *Penicillium* spp., *Rhizopus* spp., and *Trichoderma* spp.

The diversity and dominace index, suppressive and conducive soil

Suppressive soil diversity index at 1.2304 in the lower criteria, with the dominance index of 0.6677, the highest prevalence was found in *Fusarium* spp. amounting to 44.22%, while on the ground conducive diversity index reached 1.2811, with 0.6838 dominance index. The highest prevalence achieved by *Fusarium* spp. amounting to 43.06%. Diversity index showed variations habitat micoflora contained in cabbage plants in three locations. Diversity index of 1.2304 and 1.2811 showed a low index, which is due to the dominance of other mikoflora which in this case is *Fusarium* spp. amounted to 44.22% and 43.06% (Table 1).

Table 1. Diversity and dominance index, and prevalence of soil suppressive and conducive

Fungal name	Suppressive soil			Condussive soil		
	Population ($x10^3$cfu/g)**	Prevalence (%)	H'	Population ($x10^3$cfu/g)	Prevalence (%)	H'
Aspergillus spp.	139	23.28		122	28.71	
Aspergillus flavus	2	0.34		2	0.47	
Aspergillus niger	3	0.50		1	0.24	
Fusarium spp.	264	44.22	1.2304	183	43.06	1.2811
Penicillium spp.	9	1.51		25	5.88	
Rhizopus spp.	9	1.51		2	0.47	
Trichoderma spp.	171	28.64		90	21.18	
Jumlah	597			425		

**Highly significant with Chi kuadrat test, H' = diversity index

The low index of diversity caused by many fungi which dominates in soil suppressive or conducive ground, in this case is *Fusarium* spp., In addition to *Fusarium* spp., There is also the fungus *Trichoderma* spp. which dominates both the suppressive soil at 28.64. *Trichoderma* spp. mycoparasite fungus known as antagonistic to other fungi in the soil.

There are several mechanisms of suppression biology of plant diseases include: (1) antagonism, the ability of microbes specifically advantageous to produce antibiotics that can kill the organism pathogen, (2) competition for nutrients and energy, which in some cases pathogenic organism is a competitor that is ugly in conjunction with beneficial microbes. In nutrition and energy involved in the substrate, (3) competition for colonization of the roots, it is associated with root disease. Some microbes are useful have the ability to hold the colonization of plant roots before pathogen can infect, so that the roots are protected, (4) Induced Systemic Resistance (ISR) or Systemic Acquired Resistance (SAR), is a mechanism by which the gene suppressive actively working to facing the disease-causing pathogens, plant genes that encode resistance compounds worked well due to the stimulation of beneficial microbes and pathogens that cause disease (McKellar and Nelson, 2003; Alexander, 2006; Singh and Singh, 2008).

CONCLUSION

Based on results and discussion can be summarized as follows: The index of diversity both on land and conducive suppressive of 1.2304 and 1.2811 respectively, and the index of dominance on the ground suppressive and conducive row by 0.6677 and 0.6838. Prevalence mikoflora the suppressive soil amounted to 44.22% and 43.06% ground conducive all dominated by *Fusarium* spp. Microbes as a potential antagonist controlling *P. brassicae* was *Trichoderma* sp. Based on the analysis in the suppressive soil as much as 171 x 10^3 cfu/g soil, higher than on the ground conducive to 90 x 10^3 cfu/g soil.

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