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MYCOFLORA POTENTIAL IN RIZOSPHER AS BIOLOGICAL GENTSTOCONTROLWILTDISEASES ON PEPPER PLANT
(Capsicum frutescensL.)

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Abstract:-
The cause of wilt disease in pepperis Phytophthora capsicifungi, which attacked the pepper plantation area in Kertalangu Village, East Denpasar Sub-district, Denpasar-Bali. The results showed that the fungi on conducive soil were Phytophthora sp., Aspergillussp., and Neurosporasp. With the highest prevalence attained by Neurosporasp. of 80%, while on suppressive soil found mycellia sterillia fungus, Fusariumsp., Neurosporasp. Aspergillussp., Penicilliumsp., Mucorsp., and Trichodermasp. With the highest prevalence achieved by Penicilliumsp. By 27%. The antagonistic fungus found only in suppressive soils isthe fungus Neurosporasp., Trichodermasp., Aspergillussp., Penicillium sp., and Mucorsp. each with a percentage of resistance of 67.78 ± 1.6%, 68.52 ± 2.62%, 75.93 ± 2.62%, 68.77 ± 5.43%; and 67.59 ± 3.82%. The highest inhibition abilityis achieved byAspergillussp.

Keywords:- *Wilt disease, suppressive soil, conducive soil, antagonist, and inhibitionability.*

BACKGROUND

Diseases caused by the Phytophthoraspecies are thought to cause 90% of crown rot and woody plants, but lack of knowledge about how Phytophthora isolates often show negative results and hence other pathogens such as Fusarium, Pyhthium, Rhizoctoniaand nematodes often cause root rot and crown (Tsao, 1990). Phytophthora have been reported to cause diseases of blight, stem cancer, heart rot, fruit rot and root rot in various ranges of plant host species. Information on the distribution of various species of Phytophthora is present, and the transmission and development of the disease is still felt less. The approach strategy of future studies to control Phytophthora disease is still urgently needed (Thanh et al., 2004).

Land favorable for disease expression is conducive soil, while being able to suppress plant pathogens is called suppressive soil. In principle, the success of suppression due to antagonistic population rise of bacteria, fungi and actinomycetes. The soil capable of suppressing Phytophthora has been reported in ornamental plants and natural forests where often other soil pathogens are also depressed. The direct lysis of hyphae and inhibition of chlamydosporagermination from P. cinnamomihas been observed in suppressive soil. Emphasis is complemented by the soil antagonist activity of the soil that can produce antibiotics against Phytophthora. There are also a number of microorganisms that are hyperparasitic to the oospores of Phytophthora (Halsall, 1982).

Phytophthora capsicioccurs worldwide and causes root rot and crown rot as well as blight on leaves, fruit and stems in chili, tomato, and cucumber.These pathogens produce different types of propagules which include infection and spread. Zoospores are short lived propagules that survive for short periods, generally from day to week. Instead sporangia and hyphae (vegetative stages of pathogens) survive in soil between 4 to 8 weeks. Oospores are propagules surviving through the seasons and persisting for long periods (Larkin etal., 1995). Chili pepper (Capsicum annumL.), one of the most widely planted vegetables, is susceptible to root rot caused by P. capsici, and this disease can lead to yield loss (Sang et al., 2008).

MATERIAL AND METHODS

Place and time of research

The research was carried out in two stages: first stage of rhizosphere land survey on healthy cayenne plant in KertalanguVillage, East Denpasar, and stage II doing research activities in laboratory including Plant Disease Science Laboratory and Biotechnology Laboratory, Faculty of Agriculture Udayana University. The study was conducted from preparation to preparing reports from April 2018 to November 2018.

Sampling of Sample Land

The sample soil was taken from the rhizosphere of healthy and sick pepper plants located in Kertalangu Village, East Denpasar. The soil as the sampling site is diagonally determined, taking five points, one in the diagonal intersection and four in the middle of the diagonal line. One sample of chili pepper rhizosphere was taken four holes, each weighing 100 g, then mixed, placed in a plastic bag, inserted in an ice box. Before the soil analyzed thesample is included in the refrigerator, for 24 hours.

Dilated Dilution

Each sample soil taken 10 g of soil was diluted with 90 ml of sterile water, dilution was continued to 10-3. A total of 1 ml of dilution water is placed in a Petri dish previously filled with potato dextrose agar medium (PDA) plus antibiotic (antibacterial) levoplaxasin 250 mg/liter (w.v). Rhizosphere land fungus colonies will grow after two days, then counted the number of colonies with units of colony forming unit (cfu). Each colony was purified and transferred to a new Petri dish.

Prevalence of Isolates

The prevalence of isolates can be calculated by knowing the frequency of isolates for each Petri dish divided by all isolates in 100% Petri dishes. The magnitude of the prevalence of isolates can illustrate that there are isolates that dominate habitat in the rhizosphere, this can be known with the highest prevalence value.

Determining Diversity and Domination Indices

The diversity and dominance of contaminant fungi can be determined by calculating the Shannon-Wiener diversity index (Odum, 1971) and soil microbial dominance calculated by calculating the Simpson index (Pirzan and Pong-Masak, 2008).

(1) Index of microbial diversity

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

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

Where:
H’ = Diversity index of Shannon-Wiener

S = Number of genera
Pi = ni/N as the proportion of species to i (ni = total number of individuals total microbial type i, N = total number of individuals in total n)

The criteria used to interpret the diversity of Shannon-Wiener (Ferianita-Fachrul et al.,2005) are: H'value <1, meaning low diversity, H' value 1 -3 means diversity is moderate and H 'value> 3 means diversity pertained high.

(2) Dominance index
The soil microbial dominance index was calculated by calculating Simpson index (Pirzan and Pong-Masak, 2008), with the following formula:

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

Where:
C = Simpsonindex
S = Number of genera
Pi = ni/N as the proportion of species to i (ni = total number of individuals total microbial type i, N = total number of individuals in total n)

Furthermore, the species dominance index (D) can be calculated by a 1-C formulation (Rad et al. 2009).The criteria used to interpret the dominance of the soil microbial type are: close to 0 = low index or lower domination by one microbial species or no species that extreme dominates other species, close to 1 = large index or tends to be dominated by some microbial species (Pirzan and Pong-Cook, 2008).

Inhibition ability test
All rhizosphere fungi isolates were found to be tested for theirinhibitory resistance to Phytophthora capsici. with the dual culture method (in one Petri contained more than one isolate) grown in a Petri dish. Resistor power can be alculated using the following formula: Colony diameter P. capsici. in a single cultureminus the diameter of the colony P. capsiciin dual culture divided by the diameter of colony P. capsici. in a single 100% medium (Dollar, 2001; Mojica-Marin et al., 2008).

RESULTS AND DISCCUSSION
Diversity, Domination Index and Prevalence
Types of fungi found in soil healthy plant habitats (suppressive) are mycelia sterile as much as 3 isolates, Fusariumsp. 3 isolates, Neurosporasp. as many as 9 isolates, Aspergillussp. 9 isolates, Penicilliumsp. as many as 12 isolates, Mucorsp. 6 isolates and Trichoderma sp. as many as 3 isolates. While on soil habitat of sick plants (conducive) found Phytophthorasp. as many as 6 iolat, Aspergillussp. 3 isolates, and Neurosporassp. 36 isolates (Table 1, Fig.1).

Table1. Type of fungus that found in conducive and suppressive soil

Name of fungus that found at conducive soil	Number of fungus (isolate)	Prevalence	Name of fungus that found at suppressive soil	Number of fungus (isolate)	Prevalence
Phytophthora sp.	6	0,13	Miselia sterilia	3	0,07
Aspergillus sp.	3	0,07	*Fusarium* sp.	3	0,07
Neurospora sp.	36	0,80	*Neurospora* sp.	9	0,20
			Aspergillus sp.	9	0,21
			Penicillium sp.	12	0,27
			Mucor sp.	6	0,13
			Trichoderma sp.	3	0,07
Total	45			45	
H' = 2,0002			H' = 3,179		
D = 0,817			D = 0,338		

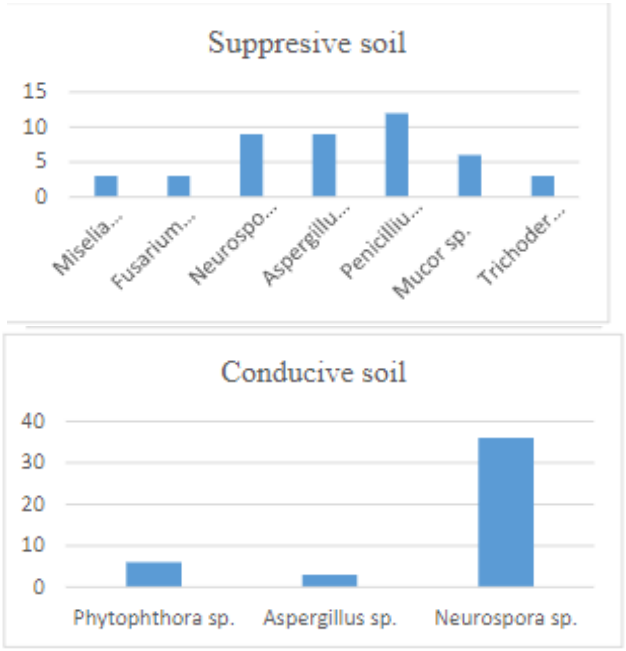

Figure 1. Number of fungus that found at suppressive soil (left) and conducive soil (right)

In suppressive soil found many 7 types of fungus the highest prevalence achieved by Penicilliumsp. of 27%, while on conducive soil found 3 types of mushrooms with the highest prevalence achieved by Neurosporasp. by 80%.The quantity of diversity index and dominance index on suppressivesoil are 2.0002 and 0,817 (Table 1). This means that the unstable environment (because the diversity index <3) if one of the species experiences death will have an effect on the other population, plus dominance is close to 1, there is a dominance of fungus that develops in that environment, Penicilliumsp. Diversity index and dominance on conducive soil as listed in Table 1. The diversity index achieved in the relatively stable conducive soil is 3.179, with the dominance index of 0.338, although the large population of the fungus Neurosporasp. but this is covered by the small population of Aspergillus sp.

Inhibition Ability of Antagonist In Vitro

Based on the result of the stratified dilution found in rhizosphere habitat of healthy plants (suppressivesoil) colony formed mean 14.13 x 105cfu, whereas on the soilhabitat (conducive soil) colony formed average 8,4 x 105cfu. Based on the data above proves that on suppressive soil the amount of microorganism is higher than on conducive soil.

The results of the separation of micoflorain suppressive soil found 5 types of fungi that indicate the inhibitionability of pathogen (Phytophthora capsici) (Fig.2). these include: Neurosporessp. With inhibitory power of 67,78 ± 1,6%, Trichodermasp. of 68.52 ± 2.62%, Aspergillussp., of 75.93 ± 2.62%, Penicilliumsp. equal to 68,77 ± 5,43% and Mucor sp. amounted to 67.59 ± 3.82%. The most visible colonies of Penicilliumsp. With a colony count of 36 x 105cfu (Table 2).

Table2. Inhibition ability of fungus that found on suppressive soil

Name of fungus	Number of colony (cfu)	Average (%)
Neurospora sp.	18 x 105	67.78 ±1.6
Trichoderma sp.	12 x 10^5	68.52 ± 2.62
Aspergillus sp.	12 x 10^5	75.93 ± 2.62
Penicillium sp.	36 x 10^5	68.77 ± 5.43
Mucor sp.	24 x 10^5	67.59 ± 3.82

The magnitude of inhibition performed by each fungus varied from 67.59 ± 3.82% to 75.93 ± 2.62% (Table 2). This is largely determined by the fungal strain, in which the ability to inhibit pathogens can be competitive, except the Trichodermafungus whose inhibition occurs by antibiosis and hyperparasitic.

Neurosporasp. This fungus is widely used as oncom which is useful for animal feed such as N. crass a and N. sitophila(Kanti and Sudiana, 2016). In the asexual part of its life cycle, the haploid asexual spore (conidial) growth and growth produce the bifurcated branching (hifa) mass, which is a colony. The hyphae do not have transverse walls so the colony is a single cell containing many haploid nuclei. Millions of conidia from air hyphae, multinucleate macroconidia and uninucleate microconidia, as well as this appear and recur in the asexual cycle if they get the appropriate substrate.

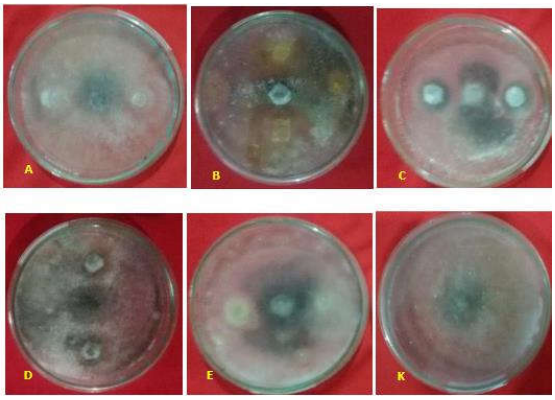

Figure 2. Inhibition ability of antagonist fungi that found on suppressive soil in vitro, (A) Neurosporasp., (B) Trichodermasp., (C) Aspergillus sp., (D) Penicillium sp., (E) Mucor sp., and(K) control (pathogen)

Trichoderma sp. is an antagonistic fungus against Colletotrichum capsici, Fuasriumsp., and Sclerotium rolfsii. The highest inhibition was achieved with C. capsiciof 68.2%, followed by Fusarium sp. of 53.9% and the lowest inhibition was achieved with S. rolfsiiof 35.5% (Alfizar et al., 2013). According to Amin et al. (2010), states that Trichodermaspp. Which was tested for its ability to inhibit the soil-borne pathogens of some vegetable samples of Rhizoctonia solani(isolated from tomato plants), Sclerotium rolfsii(the cause of tomato rot) and Sclerotium sclerotiorumin vitro, T. viride(Tv -2) the highest inhibition (71.41%) in the case of R. solani followed by T. viride(Tv-1) and T. harzianum(Tv-1) with successive inhibition of 65.71% and 60.51%.

Aspergillussp. can be used as biological agents to deal with soil pathogens. Success in controlling soil pathogens using A. nigerhas been implemented. The investigation results show the spectrum and mechanism of isolate A. nigeris an antibiosis against six fungi of soil pathogens in vitro(Patibanda and Sen, 2007).

Penicillium funiculosumhas been evaluated in greenhouses for its ability to suppress Phytophthoraroot rot from azalea (Rhododendronspp.) And sweet orange (Citrus sinensis) as measured by shoot and root growth (Fang and Tsao, 1995). According to Sempere and Santamarina (2010) states that Penicillium oxalicumfaces Alternaria alternatarice fungus under conditions of temperature, water activity, and culture medium. Microscopic analysis showed that P. oxalicumwas a mitocharasite against A. alternata. Anatogonistpenetrates into A. alternataand breaks its conidiophoreand conidia. While Mucor sp. used to detox the gadung tubers (Dioscorea hispida Dennst) through the process of permentation (Sasongko, 2009). Mucorsp. also aflatoxin B1 producer in Flores (Wange et al., 2012).

CONCLUSION

Fungi on conducive soils found were Phytophthorasp.Aspergillussp, and Neurosporasp. With the highest prevalence attained by Neurosporasp. of 80%, while on suppressive soil found mycelia sterillia, Fusariumsp., Neurosporasp. Aspergillussp., Penicilliumsp., Mucorsp., and Trichodermasp. With the highest prevalence achieved by Penicilliumsp. by 27%. The diversity (H ') and dominance (D) indexes were achieved at 2,0002, and 0.817, while the suppressive soils were 3.179 and 0.338. The antagonistic fungus found only in suppressive soils is the fungus Neurospora sp., Trichodermasp., Aspergillus sp., Penicilliumsp., and Mucorsp. each with a percentage of resistance of 67.78 ± 1.6%, 68.52 ± 2.62%, 75.93 ± 2.62%, 68.77 ± 5.43%; and 67.59 ± 3.82%. The highest percentage resistance is achieved by Aspergillussp.

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