Sensitivity of Colletotrichum Capsici Isolated from Chili Pepper (*Capsicum annum*) against Synthetic Fungicides

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Abstract: The sensitivity of *Colletotrichum capsici* isolated from chili pepper (*Capsicum annum*) againts synthetic fungicide (benomyl and hexaconazole) was studied. The aim was to determine the effect of the fungicide to the growth of *C. capsic*i. Completely factorial randomized design was used, the first factor was *C. capsici* and the second was benomil and hexaconazole at doses 0, 250, 500, 750 and 1000 ppm. Results showed that both benomil and hexaconazole inhibit the growth of *Colletotrichum capsici*, particularly doses at 750 and 1000 ppm showed the highest mycelial inhibition.

1 INTRODUCTION

Chili pepper (*Capsicum annuum* L.) is one of the horticultural products and become the priority strategic program in of the Ministry of Agriculture, Indonesia Republic in 2015-2019. Based on Badan Pusat Statistik (2017), national chili pepper yield in 2016 decreased sharply 8.47 tons/ha with an area of 123,404 ha. According to Than et al. (2008) and Kim et al. (2008) reported that *Colletotrichum capsici* was the main pathogen on chilli pepper and cause a negative impact on reducing economic value and chili production, The infections caused by *C. capsici* reaches 50% (Prathiba et al. (2013).

The use of fungicides continuously on farm to control pathogenic fungi might have negative impact on reducing the sensitivity of the pathogenic genes. The chemical compounds of fungicide such as Cu-hydroxide, diphenoconazole, mankozeb, maneb, chlorotalonil, and propineb that continuously used to control pathogenic fungi lead to increase fungal resistance (Moorman and Lease, 1992; Suganda 2001; Ziogas et al. 2005). Kumar et al. (2007) reported that the sensitivity of *C. gloeosporioides* isolated on mangoes was caused by the presence of chemical compounds in fungicide. *Colletotrichum gloeosporioides* commonly found on chili pepper.

The use of fungicide to control the pathogenic fungi are effective in inhibiting fungal growth, however, the side effect of the chemical compounds to the fungi required to be studied. The aims of the recent study was to determine the effect of synthetic fungicide containing benomyl and hexaconazole as an active compounds to control the mycelial growth of *C. capsici* isolated from chili pepper.

2 MATERIALS AND METHOD

2.1 Preparation of Fungal Isolates

The research was conducted from March to September 2019 in Microbiology and Biotechnology Laboratory, Faculty of Biology, Medan Area University. *Colletotrichum capsici* used in this study was isolated from chili pepper as culture collection of Microbiology and Biotechnology Laboratory, Medan Area University. The fungal isolate was subcultured in potato dextrose agar (PDA) and incubated for 5 days (29^{o}C).

2.2 Determination Sensitivity of *C. capsici* on Fungicide Compounds

The sensitivity of *C. capsici* was determined by calculating the relative resistance level (I) to the diameter colony. The concentration of fungicide used based on the manufacturer's *i.e* hexaconazole (1000 ppm) and benomil (1000 ppm). The relative resistance of the compounds used at doses 250, 500, 750 and 1000 ppm. Three replications were used for each treatment. Each dose of the compound was

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made by mixing the fungicide in PDA. Each compound was homogenate by adding 10 ml sterile distilled water before it was added to the PDA at 40-45°C and poured into a sterile petri dish (9 cm diameter). Fungal mycelia containing PDA medium (0.5 cm diameter) was placed into the middle of the petri dish containing PDA plate. The plates were incubated for 7 days (29°C). The percent reduction of colony was determine according to Kumar et al. (2007) as follows:

$$I = \frac{(C-T)}{C} x100\%$$

Where:
I = Percent reduction in growth of fungal,
C = Radial growth (mm) of control,
T = Radial growth (mm) of treatment

2.3 Determination the Resistance of *C. capsici* to Benomyl and Hexaconazole

The resistance of *C. capsici* to benomyl and hexaconazole was determined by the method of repeated subculture which starts from a relative inhibitory concentration> 90% (very sensitive) through poisoning of growing media. Isolates from concentration treatments with I > 90% were sub-cultured back to new media with the same concentration. Measurement of the diameter to determine the (I) is done by the method as in the previous experiment. Subculture experiments were stopped when there was a change in the level of sensitivity of each isolate to the fungicide active ingredient being tested.

2.4 Determination of *C. capsici* Sensitivity Rate for Fungicide Compounds

The sensitivity level of *C. capsici* isolates to fungicide active ingredients was determined based on the level of relative inhibition (I) based on Kumar et al (2007) as follows: Very sensitive (I) >90%, Sensitive : 75% < I ≤ 90%, Moderate resistance: 60% < I ≤ 75%, Resistant: 40% < I ≤ 60%, and Very resistant: I ≤ 40%.

3 RESULTS AND DISCUSSION

3.1 The Sensitivity of *C. Capsici* to Benomyl and Hexaconazole

The sensitivity of *C. capsici* was determined from the level of relative inhibition of the fungicide on the growth of the isolate colony diameter of *C. capsici.*. The statistical analysis, both single factors, species or isolates and types of active ingredients, interactions on the sensitivity level of *C. capsici* based on their relative level of resistance, can be seen in Table 1.

Table 1 shows the fungicide have a significant effect on *C. capsici*. Benomil in the 1000 ppm showed no growth of *C. capsici* on observations of 2, 4 , 6 , and 8 days. Administration of hexaconazole in the 4 days treatment of 1000 ppm showed no growth of *C. capsici* on observations at day 2, day 4, day 6 and day 8. This indicates that dose at 1000 ppm inhibit the growth of the pathogenic fungi. It seem that the growth of *C. capsici* at dose 750 ppm was inhibited. According to Andriani and Desta (2017) benomyl is an active ingredient of systemic fungicide by specifically targeting to disrupt mitosis β-tubulin and cell division.

Fungicide with specific compounds showed high response in inhibiting the growth of the fungal pathogen. The fungicides prevents fungal infection by forming a barrier layer on the surface of the plant (Peres et al. 2004).

3.2 Level of Relative Barriers of *C. capsici* Pathogens to Benomyl and Hexaconazole

The level of relative inhibition of *C. capsici* pathogens against benomyl and hexaconazole shows different results as indicated by the doses and type of fungicide (Table 2).

Potential development of *C. capsici* isolates resistance to fungicide active ingredients was selected based on relative inhibitory values> 90%. The test of the potential for resistance development using (I) > 90% aims to determine the ability of these isolates to adapt in developing resistance. Table 2 shows that doses of 750 ppm and 1000 ppm are the best treatment for the level of relative pathogenic inhibition.

Table 1: Relative levels of *C. capsici* to benomyl and hexaconazole.

Treatments	Incubation time (days) / reduction colony diameter (%)			
	2	4	6	8
Control	3.78^{d}	4.73^{c}	6.40^{c}	7.07^{c}
Colletotrichum × benomyl 250 ppm	0.95^{c}	1.02^{b}	1.12 b	1.03^{b}
Colletotrichum × benomyl 500 ppm	0.50^{b}	0.52^{ab}	0.60^{b}	0.87^{ab}
Colletotrichum × benomyl 750 ppm	0.00^{a}	0.28^{ab}	0.32^{a}	0.42^{a}
Colletotrichum × benomyl 1000 ppm	0.00^{a}	0.00^{a}	0.00^{a}	0.20^{a}
Control	3.78^{c}	3.82^{d}	4.00^{c}	4.03^{d}
Colletotrichum × hexaconazole 250 ppm	0.95^{b}	0.70^{c}	0.85^{b}	0.87^{c}
Colletotrichum × hexaconazole 500 ppm	0.35^{a}	0.45^{bc}	0.52^{b}	0.72^{bc}
Colletotrichum × hexaconazole 750 ppm	0.00^{a}	0.10^{ab}	0.27^{ab}	0.37^{ab}
Colletotrichum × hexaconazole 1000 ppm	0.00^{a}	0.00^{a}	0.00^{a}	0.18^{a}

Note: Numbers followed by same letters not significantly different (P≤0.05) according to Duncan's multiple range test (DMRT)

Table 2: Sensitivity of *C. capsici* on concentration of fungicide compounds (benomyl and hexaconazole).

Compounds of fungicide (ppm)/*Colletotrichum capsici* sensitivity									
Benomyl					Hexaconazole				
0	250	500	750	1000	0	250	500	750	1000
0.00 (vr)	79.19 (s)	85.37 (s)	95.52 (vs)	100 (vs)	0.00 (vr)	83.10 (s)	86.43 (s)	91.69 (vs)	100 (vs)

Note: vr = very sensitive; s = sensitive

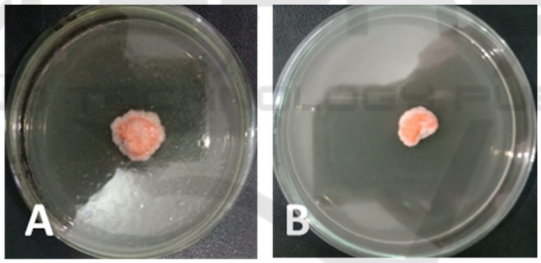

Figure 1: Colletotrichum capsici in PDA contain benomyl 1000 ppm (a) and hexaconazole 1000 ppm (b) after 8 days incubation (29°C).

The growth of colony diameter had a slightly difference in each culture during 8 DAF (days after application). The relative level of inhibition of the active ingredient of benomyl fungicide on the growth of C. capsici (Figure 1).

The level of relative inhibition to the growth diameter benomil changes in all observations. Development of pathogenic resistance to benomyl active ingredients at doses of 750 and 1000 ppm occurs more slowly. This indicates that the active ingredients are generally non-systemic which has a low risk for developing pathogenic fungal resistance.

Benomyl is an active ingredient with specific mechanism that interfere with mitosis β-tubulin and cell division Djojosumarto (2008). Fungicides with multisite mode of action mechanism by general non-systemic which has a low risk for developing fungal resistance to the active ingredient (Kumar et al. (2007). The resistance involves gene mutations. Fungal resistance to the fungicide compounds occurred while the pathogenic fungi are exposed continuously to the compounds (Andriani and Desta (2017). *Colletotrichum gloeosporioides* tend to be sensitive to fungicidal compounds even at low concentrations.

4 CONCLUSIONS

From the results of the study it can be concluded that the administration of benomil at doses of 750 ppm and 1000 ppm is the best dose in inhibiting the growth of *C. capsici* which is 0.00 mm and shows a significant effect on the relative inhibition level of the test pathogen colony.

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