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Selection Of Endophytic Fungus From Shallots As Antagonists Of *Colletotrichum gloeosporioides*

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INTRODUCTION

- Shallot is a vegetable crop with high economic value, and one of the important commodities that are cultivited by Indonesian people.
- One problem in the cultivation of shallots is the presence of pests and disease.
- Some kinds of pests and diseases that attack shallot plants are: *Spodoptera exigua*, *Liriomyza* sp, Moler disease (*Fusarium oxysporum*) , Purple blotch (Alternaria porri), Anthracnose disease (*Colletotrichum gloeosporioides*)

INTRODUCTION

The symptoms appear initially on the leaves as water soaked pale yellow spots, which spreads lengthwise covering entire leaf blade. The affected leaves shrivel and droop down.

CONTROL

- Since the pathogen survives on crop debris, sanitation and destruction of infected crop debris helps in reducing the disease.
- Crop rotation
- Using Fungicides
- use of biological agents that are antagonistic to the disease causing pathogen such as endophytic fungi
- Endophytic fungi are fungi that live inside plant tissues healthy and do not cause interference to these plants,

The objectives of this experiment were to obtain endophytic fungi that have the potential as biocontrol agents against the fungus *C. gloeosporioides*

Material & Methods

Isolation of *C. gloeosporioides*

The samples identified shallot leaf experiencing symptoms of anthracnose were collected. Leaves sample were cut with small size and planted on PDA media. The media was incubated at room temperature until the fungus mycelium grew for 3-7 days. Mycelium is purified based on the colour of the colony on PDA to obtain pure fungus colonies. Identification of fungi based on morphological and microscopic characteristics

Isolation of endophytic fungi

Plant samples from land shallot agricultural healthy were collected. The sample part is the roots, bulb, stems, and leaf cuted separately and washed with water then dried. Each sample was cut again until the size of 1 cm. The samples were then sterilized with 70% alcohol for 1 minute, soaked in 1.25% NaOCl for 3 minutes, and rinsed with sterile aquades for 3 times, then dried on sterile tissue. Sterile roots, stems, bulb and leaves are planted on MEA (Malt Extract Agar) media

Antagonistic tests

Direct inhibition test

Fungal isolates were tested in vitro for their antagonistic activity against *C.gloeosporioides* using the direct opposition (Dual culture) method. Briefly, a 5 mm mycelia disc from the margin of actively growing colony of *C.gloeosporioides* was placed at about 3 cm from the wall of a 9 cm PDA plate and at the opposite side, a similar sized disc of the fungal isolate was placed. Plates were incubated at 25 ± 2 ◦C and four replicates were used for each fungus tested. Control tests were also carried out using *C.gloeosporioides* alone. For the estimation of the growth inhibition percentage, the radial growth of *C.gloeosporioides* with each of the isolated fungi and control plates was measured and recorded consecutively for seven and 14 days

Antagonistic tests

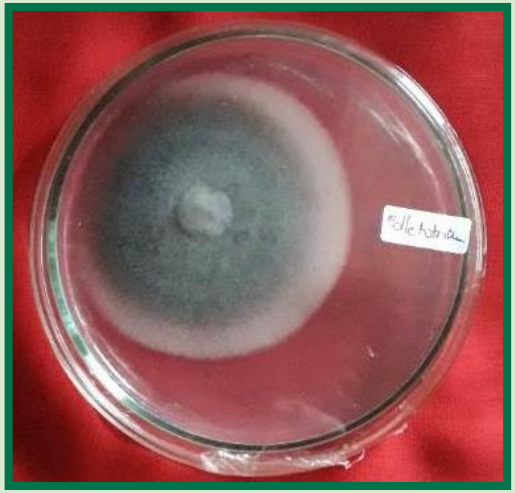
Volatile compounds test

Endophytic fungi was placed on PDA Petri dishes (9 cm) and mycelia of C.gloeosporioides paced another Petri dishes. Plates containing the phytopatogenic fungus were placed inverted on top of each of the isolated endophytic fungus. The top was sealed with parafilm and adhesive tape to prevent diffusion of volatiles. The growth of *C.gloeosporioides* in each of the plates containing a different fungus was compared with a control placed inverted in a plate containing only PDA medium. Four replicates were used. After seven and 14 days of incubation the diameters of the pathogen colonies were measured and the percentage of inhibition was calculated

RESULTS

Isolates	the percentage of inhibition (%)	
	7 dai	14 dai
S2D11	81.80 a	88.97 a
S2U31	64.52 b	78.00 bc
S2B41	62.74 bc	75.92 bcd
S1D31	62.73 bc	79.02 b
S1A23	62.68 bc	75.87 bcd
S1A11	59.96 bcd	73.20 defg
S2U34	59.10 bcd	74.83 cde
S1A41	59.04 bcd	71.17 fghi
S2U32	57.25 cde	74.33 def
S1U13	57.12 cde	69.60 hijk
S1B41	56.39 cde	72.24 efgh
S1A22	53.55 def	72.73 defgh
S1B24	51.69 efg	70.61 ghij
S1U14	49.84 fg	67.47 jk
S1A25	48.12 fg	66.93 k
S1A21	47.19 fg	69.60 hijk
S1B11	46.39 g	68.05 jik
S1D13	38.12 h	58.05 l

Direct Inhibition Test




S2P1.1


S1D3.1


S2U3.1


S1A2.1


S1D1.3

Isolates	the percentage of inhibition (%)	
	7 dai	14 dai
S2D11	52.26 a	55.92 a
S1A23	37.17 b	52.47 a
S2U31	31.09 bc	26.31 c
S2U32	30.94 bc	21.71 cd
S1U13	30.87 bc	26.76 c
S1A22	30.75 bc	51.89 a
S1B11	27.59 cd	16.67 def
S1A41	24.87 cd	36.17 b
S1D31	23.58 cd	13.93 efg
S1A25	22.63 de	15.98 def
S1U14	20.95 def	27.74 c
S1B24	20.81 def	26.25 c
S1A21	19.54 defg	6.74 g
S1D13	15.25 efgh	25.66 c
S1B41	13.37 fgh	25.97 c
S2B41	12.14 gh	19.73 cde
S1A11	11.53 h	11.74 fg
S2U34	11.36 h	10.77 fg

Volatile Compounds Test

Conclusion

- In the dual culture method, 18 isolates of endophytic fungi were able to inhibit the growth of the fungus *C. gloeosporioides*. with the percentage of inhibition 58.05-88.97% at the age of 14 dai.
- By using the volatile method, the endophytic fungus was also able to inhibit the colony area of the fungus *C. gloeosporioides* with the percentage of inhibition 10.77 – 55.92%.
- The endophytic fungus S2D11 (*Trichoderma* sp) was the most effective isolate in inhibiting the growth of the fungus *C. gloeosporioides*.

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