

RESEARCH ARTICLE

Biological Control Properties of *Cyathus* spp. to Control Plant Disease Pathogens

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Abstract

The study of efficiency of *Cyathus* spp. to control plant pathogenic bacteria and fungi the results showed that some *Cyathus* spp. effectively control plant pathogenic bacteria, such as *Pectobacteria* sp., *Pseudomonas* sp., *Ralstonia solanacearum* and *Xanthomonas campestris* pv. *vesicatoria* *in vitro*. The most effective to control *Pectobacteria* sp. was *C. striatus* KKU4 with 0.62 cm clear zone diameters. Therefore *C. striatus* KKU6 could control *Pseudomonas* sp. with 1.34 cm clear zone diameters, *C. pallidus* KKUITN2/KKULN2 could control *R. solanacearum* with 0.85 cm clear zone diameters and *C. striatus* KKU 5 was the most effective to control *X. campestris* pv. *vesicatoria* with 0.29 cm clear zone diameters. The study of antagonistic activity against plant pathogenic fungi by dual culture technique, the results showed that some species could control *Fusarium* sp. including *C. striatus* KKU3 and *C. earlei* KKULP1 with 47.94% and 56.0% inhibition, respectively when placing zero and four days before *Fusarium* sp. *C. stercoreus* KKUITP2/KKULP2 and KKUITP3/KKULP3 could control *Pythium aphanidermatum* when placing before *P. aphanidermatum* for four days. None of the isolates could control *Rhizoctonia solani* and *Sclerotium rofsii*.

Keywords: Biological control, Bird's nest fungi, *Cyathus*.

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INTRODUCTION

Cyathus species are saprobic since they obtain nutrients from decomposing organic matter. They usually grow on decaying wood or woody debris, on cow and horse dung, or directly on humus - rich soil. The genus *Cyathus* Haller belongs to the family *Nidulariaceae*, which is included in the agaricoid clade of Basidiomycota^{1,2} and it is morphologically characterized by a three-layered bell or vase-shaped basidiomata with lenticular structures (peridioles) fixed to the inner wall by the funicular cord. Several *Cyathus* species produce bioactive compounds, some with medicinal properties, and several lignin degrading enzymes from the genus may be useful in bioremediation and agriculture. *C. olla* has the potential of being developed into a microbial inoculant aimed at reducing the incidence of stubble-borne disease of canola. Isolates were cultured from field collected specimens and were also obtained from culture collections for morphological and molecular studies³. *Cyathus striatus* No.12 is highly active against fungi imperfecti and a variety of gram-positive bacteria, as well as against some gram-negative bacteria⁴. El-Fallal and Moussa⁵ reported that eight basidiomycetes, including *Cyathus stercoreus*, were tested to antagonize *Ralstonia solanacearum* (causal agent of brown rot disease of potato) *in vitro*. All of these fungi inhibited the growth of *R. solanacearum*, and the largest inhibition zones were recorded with *C. stercoreus* Egyptian strain and *Agaricus campester* Egyptian strain. Sutthisa and Sanoamuang⁶ studied the ability of bird's nest fungi (*Cyathus* sp.) to inhibit the mycelial growth of soil-borne plant pathogenic fungi by the dual culture technique. The results showed that *Cyathus* sp. inhibited the mycelial growth of *Fusarium oxysporum* f.sp. *lycopersici* (FOL), but did not inhibit *Pythium aphanidermatum*, *Sclerotium rolfsii* and *Rhizoctonia* sp., which had 62.22 - 63.64 %inhibition of mycelial growth and 21.84 - 22.46 %overgrowth. The culture filtrate of *Cyathus* showed no effect on FOL conidial germination but did affect the mycelium growth.

The objective of this research is to study antagonistic ability of bird's nest fungi (*Cyathus* sp.) against plant pathogenic bacteria and fungi for basic information to applied in the further research.

MATERIALS AND METHODS

Organisms

Twelve isolates of *Cyathus* spp. including *Cyathus striatus* (KKU1, KKU2, KKU3, KKU4, KKU5 and KKU6), *Cyathus berkeleyanus* (KKUNN1), *Cyathus earlei* (KKULP1), *Cyathus pallidus* (KKUITN2/KKULN2 and KKITN3/KKULN3) and *Cyathus stercoreus* (KKUITP2/KKULP2 and KKITP3/KKULP3) were obtained from Khon Kaen University Culture Collection (KKU). Plant pathogenic bacteria and fungi such as *Pectobacterium* sp., *Pseudomonas* sp., *Ralstonia solanacearum*, *Xanthomonas campestris* pv. *vesicatoria*, *Fusarium* sp., *Pythium aphanidermatum*, *Rhizoctonia solani* and *Sclerotium rofsii* were obtained from the Branch of Plant Pathology, Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Thailand.

Efficacy of *Cyathus* spp. controlling plant pathogenic bacteria

The agar plug technique was used to test the ability of *Cyathus* spp. to control plant pathogenic bacteria, such as *Pectobacterium* sp., *Pseudomonas* sp., *Ralstonia solanacearum* and *Xanthomonas campestris* pv. *vesicatoria*. Spread bacterial cell suspension (10^4 cfu/ml) on PDA plate after placing a 0.7 cm diameter agar disc of 10 days old *Cyathus* spp. at four points in a cross pattern and incubated at $28 \pm 2^\circ\text{C}$, and after 24-48 hr observe and measure clear zone. There were five replications per treatment.

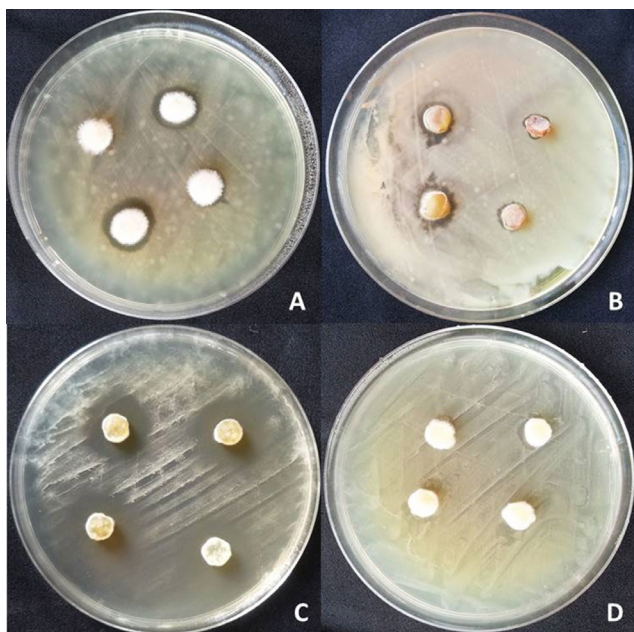
Efficacy of *Cyathus* spp. controlling plant pathogenic fungi

Cyathus spp. isolates were tested *in vitro* for their antagonistic activity against *Fusarium* sp., *Pythium aphanidermatum*, *Rhizoctonia solani* and *Sclerotium rofsii* using the dual culture technique. Two experiments were conducted: first, placing a 0.7 cm diameter disc of 10 days old *Cyathus* sp. on PDA plate and then placing a 0.7 cm diameter disc of each plant pathogenic fungi. The discs were put a 1.5 cm distance from the edge so they opposed each other on the same diagonal line. Second, placing *Cyathus* sp. four days before placing plant pathogenic fungi. Five plates were considered for each fungus-fungus interaction and daily measurements of the radial distance of *Cyathus* sp. and the plant pathogenic fungi were made. The percentage of inhibition and the percentage

Table 1 Efficacy of *Cyathus* spp. to control plant pathogenic bacteria by dual culture technique.

Isolates	Clear zone diameter (cm)			
	<i>Pectobacterium</i> sp.	<i>Pseudomonas</i> sp.	<i>Ralstonia solanacearum</i>	<i>Xanthomonas campestris</i> pv. <i>vesicatoria</i>
<i>C. striatus</i>				
KKU1	0.27±0.06 de	0.69±0.13 de	0.31±0.03 g	0.23±0.01 c
KKU2	0.38±0.14 c	0.91±0.16 b	0.71±0.23 bc	0.23±0.10 c
KKU3	0.21±0.01 e	0.65±0.05 e	0.29±0.04 g	0.26±0.01 ab
KKU4	0.62±0.10 a	0.66±0.11 e	0.58±0.09 de	0.00±0.00 d
KKU5	0.51±0.08 b	0.96±0.18 b	0.40±0.09 fg	0.29±0.05 a
KKU6	0.00±0.00 f	1.34±0.13 a	0.53±0.09 e	0.24±0.06 bc
<i>C. berkeleyanus</i>				
KKUNN1	0.28±0.03 de	0.29±0.13 g	0.33±0.22 g	0.00±0.00 d
<i>C. pallidus</i>				
KKUITN2/KKULN2	0.21±0.06 e	0.38±0.10 fg	0.85±0.17 a	0.00±0.00 d
KKUITN3/KKULN3	0.33±0.10 cd	0.43±0.10 f	0.83±0.09 ab	0.00±0.00 d
<i>C. earlei</i>				
KKULP1	0.27±0.09 de	0.78±0.05 d	0.69±0.23 cd	0.24±0.03 bc
<i>C. stercoreus</i>				
KKUITP2/KKULP2	0.00±0.00 f	0.66±0.14 e	0.00±0.00 h	0.00±0.00 d
KKUITP3/KKULP3	0.30±0.10 d	0.85±0.09 bc	0.48±0.01 ef	0.00±0.00 d
CV (%)	27.70	16.95	26.82	30.57

Note: Different superscripts within same column indicated significant difference ($P < 0.05$).

**Fig. 1.** Efficacy of *Cyathus* sp. controlling plant pathogenic bacteria by dual culture technique.

- A: *C. striatus* KKU4 + *Pectobacterium* sp.,
 B: *C. striatus* KKU6 + *Pseudomonas* sp.,
 C: *C. pallidus* KKUITN3/KKULN3 + *Ralstonia solanacearum*,
 D: *C. striatus* KKU3 + *Xanthomonas campestris* pv. *vesicatoria*

of overgrowth were calculated following Landum *et al.*⁷

Statistical analysis of data

All the data were subject to analysis of variance (ANOVA) and treatment mean comparisons were performed using least significant differences at P=0.05 by (LSD).

RESULTS

Efficacy of *Cyathus* spp. controlling plant pathogenic bacteria

The ability of *Cyathus* spp. to control plant pathogenic bacteria was tested by the dual culture technique. The results showed the best inhibitors included *C. striatus* KKU4 that could

Table 2. Efficacy of *Cyathus* spp. controlling *Fusarium* sp. by dual culture technique.

Isolates	Inoculation <i>Cyathus</i> spp. before pathogen			
	0 days		4 days	
	% Inhibition	% Overgrowth	% Inhibition	% Overgrowth
<i>C. striatus</i>				
KKU1	11.38±3.24 c	27.50±3.54 a	27.59±14.63 f	15.00±7.07 e
KKU2	18.22±9.11 cd	Clear zone	34.48±0.00 e	Clear zone
KKU3	47.94±23.97 a	10.00±0.00 d	44.83±0.00 bc	42.50±10.61 a
KKU4	31.85±15.93 b	20.00±14.14 bc	41.83±0.00 cd	35.00±0.00 b
KKU5	13.64±6.82 d	17.50±3.54 c	39.66±7.32 d	20.00±0.00 d
KKU6	22.76±11.38 c	12.50±3.54 d	48.28±4.87 b	17.50±3.54 de
<i>C. berkeleyanus</i>				
KKUNN1	0.00±0.00 e	0.00±0.00 e	0.00±0.00 g	0.00±0.00 f
<i>C. pallidus</i>				
KKUITN2/ KKULN2	0.00±0.00 e	0.00±0.00 e	0.00±0.00 g	0.00±0.00 f
KKUITN3/ KKULN3	0.00±0.00 e	0.00±0.00 e	0.00±0.00 g	0.00±0.00 f
<i>C. earlei</i>				
KKULP1	9.09±0.00 cd	Clear zone	56.90±2.44 a	Clear zone
<i>C. stercoreus</i>				
KKUITP2/ KKULP2	11.38±3.24 c	22.50±3.54 b	31.04±4.87 ef	32.50±3.54 b
KKUITP3/ KKULP3	9.09±0.00 cd	22.50±3.54 b	34.48±0.00 e	27.50±10.61 c
CV (%)	34.39	42.34	17.31	31.57

Note: Different superscripts within same column indicated significant difference (P < 0.05).

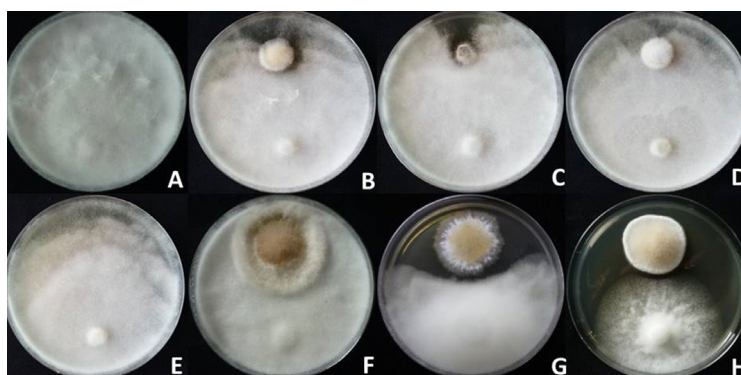


Fig. 2. Efficacy of *Cyathus* sp. controlling *Pythium aphanidermatum*. Top- Placing *Cyathus* sp. and *P. aphanidermatum* on same day. Bottom- Placing *Cyathus* sp. four days before *P. aphanidermatum*.

A,E: *P. aphanidermatum*

B,F: *P. aphanidermatum* + *C. striatus* KKU1

C, G: *P. aphanidermatum* + *C. earlei* KKULP1 D, H: *P. aphanidermatum* + *C. stercoreus* KKUITP3/ KKULP3

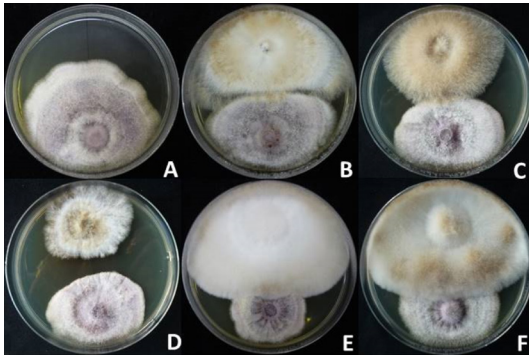


Fig. 3. Efficacy of *Cyathus* spp. controlling *Fusarium* sp.
 A: *Fusarium* sp. B: *C. striatus* KKU1 + *Fusarium* sp.
 C: *C. striatus* KU4 + *Fusarium* sp.
 D: *C. earlei* KKULP1 + *Fusarium* sp.
 E: *C. stercoreus* KKUITP2/KKULP2 + *Fusarium* sp.
 F: *C. stercoreus* KKUITP3/KKULP3 + *Fusarium* sp.

inhibit *Pectobacteria* sp. with a 0.62 cm clear zone diameter, which was significantly different from the others isolates. *C. striatus* KKU6 could inhibit *Pseudomonas* sp. with a 1.34 cm clear zone diameter, which was significantly different from the others isolates. *C. pallidus* KKUITN2/KKULN2 and KKUITN3/KKULN3 could inhibit *R. solanacearum* with 0.85 cm and 0.83 cm clear zone diameters, respectively. *C. striatus* KKU3 and KKU5 had significantly different inhibitions of *X. campestris* pv. *vesicatoria* with 0.26 cm and 0.29 cm clear zone diameters, respectively (Table 1, Fig. 1).

Efficacy of *Cyathus* sp. controlling plant pathogenic fungi

Cyathus spp. isolates were tested *in vitro* for their antagonistic activity against *Fusarium* sp., *P. aphanidermatum*, *R. solani* and *S. rofsii* using the dual culture technique. The results show that none of the isolates could inhibit *P. aphanidermatum*, *Rhizoctonia* sp. and *S. rofsii*, due to the plant pathogenic fungi mycelial growing rapidly and covered the *Cyathus* spp. colony. It was found that *C. earlei* KKULP1, *C. stercoreus* KKUITP2/KKULP2 and KKUITP3/KKULP3 could inhibit the mycelial growth of *P. aphanidermatum* with 54.31, 48.28% and 47.41 %inhibition and 20%, 21.67% and 20 %overgrowth, respectively (Fig. 2). All isolates could inhibit the mycelial growth of *Fusarium* sp. in both experiments. Except, *C. berkeleyanus* KKUNN1, *C. pallidus* KKUITN2/KKULN2 and KKUITN3/KKULN3 because they grew slowly. The most effective control of

Fusarium sp. in experiment I was *C. striatus* KKU3 with 47.94 %inhibition and 10 %overgrowth, and in experiment II it was *C. earlei* KKULP1 with 56.90 %inhibition and a clear zone (Table 2, Fig. 3).

DISCUSSION

Efficacy tests of bird's nest fungi to control plant pathogenic bacteria and fungi by the dual culture technique were performed, and we found that some isolates could inhibit *Pectobacteria* sp., *Pseudomonas* sp., *R. solanacearum* and *X. campestris* pv. *vesicatoria* with different clear zone diameters. Some isolates could control *Fusarium* sp. when both placing the bird's nest fungi and *Fusarium* sp. on the same date and when placing the *Fusarium* sp. four days before with overgrowth and clear zone mechanism. Except *C. berkeleyanus* KKUNN1, *C. pallidus* KKUITN2/KKULN2 and KKUITN3/KKULN3 because of their slow growth. Only *C. stercoreus* KKUITP2/KKULP2 and KKUITP3/KKULP3 could control *P. aphanidermatum* when placing four days before *P. aphanidermatum*. Moreover, we found that after *P. aphanidermatum* was covered with bird's nest fungi the day after that bird's nest fungi was placed it recover growth and covered *P. aphanidermatum*. None of the isolates could inhibit *Rhizoctonia* sp. and *S. rofsii* due to the plant pathogenic fungi's rapid mycelial growth that covered the bird's nest fungi colony. El-Fallal and Moussa⁵ reported that eight basidiomycetes, including *Cyathus stercoreus*, were tested to antagonize *Ralstonia solanacearum* (causal agent of brown rot disease of potato) *in vitro*. All of these fungi inhibited the growth of *R. solanacearum* and the largest inhibition zones were recorded with *C. stercoreus* Egyptian strain and *Agaricus campester* Egyptian strain. Suwanpitak⁸ investigated the antagonistic activities *in vitro* of wood rotting fungi on two important wilt pathogens (*Fusarium oxysporum* f.sp. *lycopersici* and *R. solanacearum*) in which 45 isolates of wood rotting fungi were screened individually against both pathogens using the dual culture technique *in vitro* and measuring the width of the colony growth or zone of inhibition. Bird's nest fungi (*Cyathus* spp.) isolates KK1, KK2, KK3, KK4 and Shiitake (*Lentinula edodes*) showed the best activities against *F. oxysporum* f.sp. *lycopersici*. All isolates of the bird's nest fungi, KK1, KK2, KK3, KK4 and NN inhibited the growth of *R. solanacearum* with large inhibition

zones. Moreover, the bird's nest fungi, KK1, KK2, KK3, KK4 and NN inhibited the growth of *Pythium* sp. and *Rhizoctonia solani*, but they could not inhibit the growth of *S. rolfsii*. This is consistent with our previous study on the efficiency of bird's nest fungi (*Cyathus* sp.) to inhibit the mycelial growth of soil-borne plant pathogenic fungi carried out by the dual culture technique. The results showed that *Cyathus* sp. inhibited the mycelial growth of *Fusarium oxysporum* f.sp. *lycopersici* (FOL), but did not inhibit *P. aphanidermatum*, *S. rolfsii* and *Rhizoctonia* sp. The greatest inhibitions were obtained from isolates KKU1 and KKU2 with 63.64% and 62.22 %inhibition of mycelial growth and 22.46% and 21.84 %overgrowth, respectively. The culture filtrate of *Cyathus* showed no effect on the FOL conidial germination but did affect the mycelium growth. The ability of *Cyathus* sp. to control tomato wilt disease caused by *F. oxysporum* f.sp. *lycopersici* under greenhouse conditions was done. At twenty-eight days after inoculation, KKU1 could reduce the disease incident more than KKU2. *Cyathus* sp. KKU1and KKU2 enhanced the growth of tomato that caused increasing tomato dry weight and height⁶. Lui and Zhang⁹ reported that 12 selected *Cyathus* species were tested for their abilities to produce antimicrobial metabolites. Most of them were found to produce secondary exo-metabolites that could induce morphological abnormalities in the rice pathogenic fungi *Pyricularia oryzae*. Moreover, Dong *et al.*¹⁰ study about nematicidal effect of freshwater fungal cultures against the pine-wood nematode (*Bursaphelenchus xylophilus*). The results showed that the nematicidal effect only occurred in the broken hyphal extracts and not in filtrates. The nematicide is secreted within the fungi hyphae (i.e. endotoxin). Natural and neutrally adjusted extracts of 13 fungal hyphae were pathogenic to the nematodes. They were species of *Annulatascus*, *Caryospora*, *Cyathus*, *Ophioceras* and *Phomatospora*.

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