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* KKU5 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*.
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 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 funiculcular 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 studies. _Cyathus striatus_ No.12 is highly active against fungi imperfecti and a variety of gram-positive bacteria, as well as against some gram-negative bacteria. El-Fallal and Moussa 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 Sanoamuang 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 solani_ 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_ (KKUIN2/KKULN2 and KKITN3/KKULN3) and _Cyathus stercoreus_ (KKUIN2/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 rolfsii_ 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 (10⁴ cfu/ml) on PDA plate after placing a 0.7 cm diameter agar disc of 10 days old _Cyathus_ sp. at four points in a cross pattern and incubated at 28 ± 2°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 rolfsii_ 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) | Pectobacterium sp. | Pseudomonas sp. | Ralstonia solanacearum | Xanthomonas campestris pv. vesicatoria |
|-----------------------|--------------------------|---------------------|-----------------|------------------------|----------------------------------------|
|                       |                          |                     |                 |                        |                                        |
| *C. striatus*         |                          |                     |                 |                        |                                        |
| 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           |
| *C. berkeleyanus*     |                          |                     |                 |                        |                                        |
| KKUNN1                | 0.28±0.03 de             | 0.29±0.13 g         | 0.33±0.22 g     | 0.00±0.00 d            |
| *C. pallidus*         |                          |                     |                 |                        |                                        |
| 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            |
| *C. earlei*           |                          |                     |                 |                        |                                        |
| KKULP1                | 0.27±0.09 de             | 0.78±0.05 d         | 0.69±0.23 cd    | 0.24±0.03 bc           |
| *C. stercoreus*       |                          |                     |                 |                        |                                        |
| 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 + Pectobacteria 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 (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 Cyathus spp. before pathogen | 0 days | 4 days |
|------------------|-----------------------------------------|--------|--------|
|                  |                                         | % Inhibition | % Overgrowth | % Inhibition | % Overgrowth |
| *C. striatus*    |                                         |        |        |
| 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±1.00 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±10.00 b |
| KKU5             | 13.64±6.82 b                            | 17.50±3.54 c | 39.66±7.32 d | 20.00±10.00 d |
| KKU6             | 22.76±11.38 c                           | 12.50±3.54 d | 48.28±8.47 b | 17.50±3.54 de |
| *C. berkeleyanus*|                                         |        |        |
| KKUNN1           | 0.00±0.00 e                             | 0.00±0.00 e | 0.00±0.00 e | 0.00±0.00 e |
| *C. pallidus*    |                                         |        |        |
| KKUITN2/ KKULN2  | 0.00±0.00 e                             | 0.00±0.00 e | 0.00±0.00 e | 0.00±0.00 e |
| KKUITN3/ KKULN3  | 0.00±0.00 e                             | 0.00±0.00 e | 0.00±0.00 e | 0.00±0.00 e |
| *C. earlei*      |                                         |        |        |
| KKULP1           | 9.09±0.00 cd                            | Clear zone | 56.90±2.44 a | Clear zone |
| *C. stercoreus*  |                                         |        |        |
| 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).

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**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
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 KKUIN2/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 *vivo* 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 KKUIN1, C. pallidus KKUITN2/KKULN2 and KKUITN3/KKULN3 because of their slow growth. Only C. stercoreus KKUITP2/KKULP2 and KKIP3/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 Moussa reported that eight basidiomycetes, including Cyathus stercoreus, were tested to antagonize *Ralstonia solanacearum* (causal agent of brown rot disease of potato) *vivo*. 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. Suwanpitak investigated the antagonistic activities *vivo* 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 *vivo* 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. KKU1 and KKU2 enhanced the growth of tomato that caused increasing tomato dry weight and height.

Lui and Zhang 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. 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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