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RESEARCH ARTICLE

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Field Efficacy of Antagonistic Fungi against Black Spot Disease of Chinese Kale

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ABSTRACT

Background: *Alternaria brassicicola* (Schw.) causes black spot disease, which is one of the major diseases limiting the production of vegetable crops, especially Chinese kale in Southeast Asia and Thailand. Previous pathogen management based on synthetic fungicides is expensive, toxic for humans and harmful to the environment. The current study investigated the efficiency of *Talaromyces flavus* (Klöcker) Stolk and Samson Bodhi001, *Talaromyces trachyspermus* (Shear) Stolk and Samson Bodhi002, *Talaromyces flavus* (Klöcker) Stolk and Samson Bodhi003, *Neosartorya fischeri* (Wehmer) Malloch and Cain Bodhi004 and *Neosartorya fischeri* (Wehmer) Malloch and Cain in controlling Chinese kale black spot disease caused by *A. brassicicola* under field conditions.

Methods: *A. brassicicola* and four antagonistic fungal strains were cultured separately on a PDA plate and incubated at room temperature for 14 days. Ten mL of sterile water was poured into a culture plate and the spores were gently scraped from the mycelium with a sterile loop to obtain a spore suspension and afterwards adjusted to a final concentration of 10^6 spores mL^{-1}.

Result: The results showed that spore suspensions of 10^6 spores mL^{-1} of *T. flavus* Bodhi001, *T. trachyspermus* Bodhi002, *T. flavus* Bodhi003, *N. fischeri* Bodhi004 and *N. fischeri* effectively controlled black spot disease in field trials and resulted in a significant reduction in black spot incidence compared with the unprotected control. Meanwhile, the spore suspension of *T. flavus* Bodhi001 revealed the greatest suppression of black spot incidence, causing 10.23% and 42.93% disease reduction, compared with the negative control, indicating promising preventive activity against *A. brassicicola.* Based on our results, *T. favus* Bodhi001 is a promising biological control agent (BCA) in controlling *A. brassicicola* causing Chinese kale black spot disease.

Key words: Antagonistic fungi, Biological control, Chinese kale black spot disease, *Talaromyces favus* Bodhi001.

INTRODUCTION

Chinese kale (*Brassica alboglabra*), which belongs to the Brassicaceae family, is a popular vegetable crop that provides edible leaves, stems and flowers (Rakow, 2004) and is widely cultivated in Thailand and Southeast Asia. The crop yields and quality, however, have been reduced by the plant pathogenic fungus *Alternaria brassicicola* (Schw.), which causes Chinese kale black spot disease. *Alternaria brassicicola* is a major risk to many brassicaceous crops worldwide as it can spread throughout all parts of the plant, thus increasing the costs for disease management. Plants infected by this fungus show black spot disease on the leaves, stems, or whole plant, resulting in the loss of quantitative and qualitative yields in an infected crop of about 20%-86% (Nowicki *et al.,* 2012). Disease symptoms are mainly observed on the leaves as black, dark brown spots or necrotic lesions with yellow halos surrounding the chlorotic zone at an early stage. Under favorable field conditions, the disease causes significant crop losses. In infected plant parts, the pathogen can produce an effective spore, with an associated pathogenic toxin, that can easily spread by wind, rain and insects to other field crops (Pattanamahakul and Strange, 1999; Lin *et al.,* 2011; Siciliano *et al.,* 2017). In general, the disease is found during the rainy season in successive crops in infected fields since the pathogen can survive in plant debris from one season to the next on alternate host plants (Jones, 1992; Nowicki *et al.,* 2012; Fernandez-Rodriguez *et al.,* 2015). Moreover, Thai farmers have been using synthetic fungicides as curative and protective measures to control the black spot disease pathogen in their plants. However, the continuous use of increasingly large amounts of synthetic fungicides is harmful to human health, non-target organisms, the local ecology and the environment, as well as a risk factor for the development of pathogen resistance to various fungicides (Thind, 2012).

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Presently, the growing trend toward sustainable agriculture and organic farming aims to promote the control of plant diseases by using biological control agents (BCAs) to reduce the use of synthetic fungicides. Consequently, BCAs developed by exploring antagonistic microorganisms isolated from soils, epiphytic and endophytic plants and marine microorganisms are effective substances for controlling plant pathogens causing economic crop diseases (Zhang *et al.,* 2014; Bahramian *et al.,* 2016; Jantasorn *et al.,* 2017; Dethoup *et al.,* 2018a; Komhorm *et al.,* 2021). Nowadays, many studies of BCAs and plant extracts have been conducted to assess their potential for developing new biological products that are safe and eco-friendly biofungicides for controlling plant diseases. The antagonistic fungi of the genus *Talaromyces* have multiple benefits for plant disease control; they have been used as biological agents against several plant pathogens in vegetables and crops, such as cucumber and tomato (Bahramian *et al.,* 2016; Halo *et al.,* 2019), Chinese kale (Komhorm *et al.,* 2021), cotton and potato (Naraghi *et al.,* 2012) and rice (Shen *et al.,* 2014; Dethoup *et al.,* 2018a). In our previous study, we evaluated the potential of six antagonistic fungi against *Alternaria brassicicola* causing Chinese kale black spot disease under greenhouse conditions and found that *Talaromyces flavus* Bodhi001 exhibited the greatest suppression of the development of black spot disease and reduced the disease incidence by up to 32.56%, which makes it a promising biological control agent against *A. brassicicola* (Komhorm *et al.,* 2021). However, further studies are required to test the antagonistic activity of this strain in controlling black spot disease under field conditions. Therefore, the objective of this study was to evaluate the potential of the antagonistic fungi *T. favus* Bodhi001, *T. trachyspermus* Bodhi002, *T. favus* Bodhi003 and *N. fischeri* Bodhi004 against *Alternaria brassicicola* causing Chinese kale black spot disease compared with a commercial fungicide, as assessed under field conditions.

MATERIALS AND METHODS

Antagonistic fungi

T. favus Bodhi001, *T. trachyspermus* Bodhi002, *T. favus* Bodhi003 and *N. fischeri* Bodhi004 strains were isolated from soil samples from a riparian forest at Bodhivijjalaya College, Srinakharinwirot University, Sakaeo Campus, Thailand. The isolation method used was previously reported by Jantasorn *et al.* (2016b) and Komhorm *et al.* (2021). Briefly, 1 g of soil samples was placed in a sterile test tube and incubated at 65°C for 15 min in a water bath. After incubation, the samples were transferred into Petri dishes and poured over with warm glucose ammonium nitrate agar containing 0.05% streptomycin sulfate. Then, the samples were incubated in darkness at room temperature for 3 days. Hyphal tips of pure fungi were transferred onto a potato dextrose agar (PDA) slant and maintained on PDA at 4°C in the laboratory at Bodhivijjalaya College, Srinakharinwirot University, Ongkharak Campus, Thailand.

Fungal pathogen and fungal spore suspensions

The fungal pathogen *A. brassicicola,* which causes Chinese kale black spot disease and *N. fischeri* were obtained from Prof. Dr. Tida Dethoup, Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand (Dethoup *et al.,* 2018b). Their identity was confirmed by their morphological characteristics via microscopic observation and their pathogenicity was checked according to Koch's postulates. *A. brassicicola* and four antagonistic fungus strains were cultured separately on a PDA plate and incubated at room temperature for 14 days. Ten mL of sterile water was poured into a culture plate and the spores were gently scraped from the mycelium with a sterile loop to obtain a spore suspension. The spore suspension was filtered through three layers of sterile cheesecloth under aseptic conditions and afterwards adjusted to a final concentration of 10^6 spores mL^{-1} using a hemocytometer.

Protective efficacy of antagonistic fungi against Chinese kale black spot disease in field assays

Seeds of the Chinese kale cultivar Maejo 1 were used for the field experiment. This cultivar is susceptible to *A. brassicicola*. The field experiment was performed in a Chinese kale plantation at Amphur Tha Maka, Kanchanaburi province, in the western region of Thailand during the growing season 2020-2021. The individual pot size was 1.5 × 1.5 m, with a distance of 0.1 m within and between the rows and a distance of 0.5 m between the pots, for a total of 18 rows per pot. The treatments were performed in a completely randomized design with three replicates per treatment and the design was repeated twice. Ten-day-old kale seedlings of the same height and vigour were transplanted into the pots. Thirty days after planting, the Chinese kale plants were first sprayed separately with spore suspensions of the five antagonistic fungi containing 10^6 spores mL^{-1} with 0.01% (v/v) Tween-20 per pot on both sides of the leaves. One litre of the spore suspension of the five antagonistic fungi was sprayed with separate sprayers in each treatment consisting of three pots per treatment, which were then allowed to dry for 1 h. Then, one litre of the spore suspension (10^6 spores mL^{-1}) of *A. brassicicola* was inoculated on the treated plants in each of the three pots (as the replicates) per treatment. The negative control treatment with three replicates consisted of distilled water containing 0.01% (v/v) Tween-20. Disease incidence and disease severity were determined 7 days after pathogen inoculation. Forty leaves were randomly collected and inspected for disease incidence. Disease incidence was calculated as the percentage of plants with disease symptoms and recorded as an average for the lesion area over the total surface per leaf compared with the water control treatment (Komhorm *et al.,* 2021). The disease severity was divided into six levels: 0= no lesion; 1= lesion area 1%-20%; 2= lesion area 21%-40%; 3= lesion area 41%-60%; 4= lesion area 11%-80%; 5= lesion area over 80%

(Panwar *et al.,* 2013). Iprodione (50% WP, Bayer Crop Science) was applied as a positive treatment.

Statistical analysis

The experiments were conducted twice. An analysis of variance (ANOVA) was used to evaluate and analyze the data from each experiment in different treatments and the mean data were compared by the least significant difference (LSD) with a 95% statistical significance ($p<0.05$), using the statistical program Statistix8 (Analytical Software, SXW, Tallahassee, FL, USA).

RESULTS AND DISCUSSION

Effect of antagonistic fungi against Chinese kale black spot disease in field assays

The efficiency tests of the five antagonistic fungi in controlling *A. brassicicola* causing Chinese kale black spot disease in field assays are shown in Table 1. Treatment with the spore suspension of *T. favus* Bodhi001, *T. trachyspermus* Bodhi002, *T. favus* Bodhi003, *N. fischeri* Bodhi004 and *N. fischeri* at 10^6 spores mL^{-1} tested before pathogen inoculation effectively controlled black spot disease and resulted in a significant reduction in black spot incidence compared with the water control. However, the greatest suppression of the development of black spots under field conditions occurred when the plants were treated with a spore suspension of *T. favus* Bodhi001, causing 10.23% and 42.93% disease reduction. The application of *T. favus* Bodhi001 showed an excellent inhibitory effect against the development of typical black spot symptoms caused by *A. brassicicola* compared with the other antagonistic fungi and negative control treatments (Fig 1). Besides, the plants treated with distilled water showed the highest incidence of typical black spot symptoms. The protective activities of the spore suspensions of *T. favus* Bodhi001, *T. trachyspermus* Bodhi002, *T. favus* Bodhi003, *N. fischeri* Bodhi004 and *N. fischeri* against black spot disease of Chinese kale in field assays were compared and they were found to reduce black spot incidence by 10.23%, 15.50%, 16.07%, 15.33% and 16.77%, respectively. Interestingly, we found that the treatment with *T. favus* Bodhi001 displayed the greatest suppression of black spot incidence, to the same extent as the positive control with the synthetic fungicide.

Presently, biological control agents are a promising approach to the management of plant diseases in organic crop production and sustainable agriculture, with the aim to reduce the use of synthetic fungicides. The results of this study showed that it may be possible to manage plant diseases in economically important crops, especially black spot disease in Chinese kale, efficiently by treatment with effective antagonistic fungi of *T. flavus* Bodhi001 isolates in field assays (Table 1, Fig 1). Based on the field assay results, *T. flavus* Bodhi001 displayed the highest *A. brassicicola* suppression activity. This finding is similar to that in our previous study, in which we found that *T. flavus* Bodhi001 exhibited the greatest suppression of the development of black spot disease and reduced the disease incidence by up to 32.56% under greenhouse conditions, which made it a promising biological control agent against *A. brassicicola* (Komhorm *et al.,* 2021). Moreover, the extract of *T. flavus* Bodhi001 also completely inhibited the mycelial growth of *Colletotrichum capsici*, *Colletotrichum gloeosporioides*, *Rhizoctonia solani*, *Phytophthora palmivora*, *Alternaria* sp., *Fusarium oxysporum*, *Helminthosporium* sp., *Pyricularia oryzae*, *Sclerotium rolfsii* and *Lasiodiplodia theobromae* at a concentration of 10,000 ppm under *in vitro* conditions (Jantasorn *et al.,* 2016a). However, many other studies also tested the efficacies of antagonistic microorganisms and plant extracts against *Alternaria* spp. in economic crops both *in vitro* and *in vivo* (Jantasorn *et al.,* 2016a; Jantasorn *et al.,* 2016b; Jantasorn *et al.,* 2017; El-Gremi *et al.,* 2017; El-Hossary *et al.,* 2017; Dethoup *et al.,* 2018b; Ounchokdee and Dethoup, 2020; Komhorm *et al.,* 2021). Moreover, the different strains of *Talaromyces* spp. displayed antagonistic activity by producing an antifungal compound and mycoparasitism *via* biological control in the suppression of

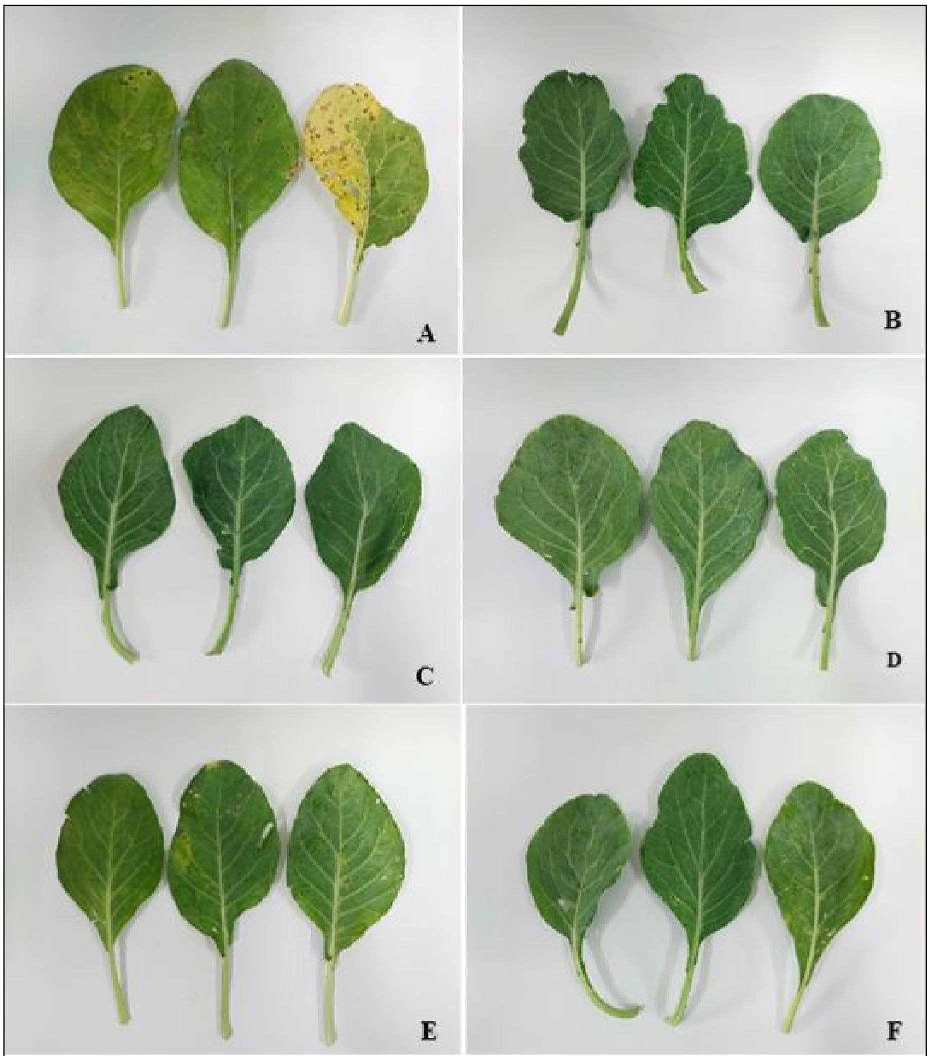
Table 1: Effect of ~~a~~ spore suspension of antagonistic fungi on disease incidence and disease reduction of Chinese kale black spot disease caused by *Alternaria brassicicola* under field conditions.

Treatments	Disease incidence (%)	Disease reduction (%)
Talaromyces flavus Bodhi001	$10.23^{b}\pm2.49$	$42.93^{b}\pm1.93$
Talaromyces trachyspermus Bodhi002	$15.50^{c}\pm3.87$	$37.67^{c}\pm3.23$
Talaromyces flavus Bodhi003	$16.07^{c}\pm4.11$	$37.10^{c}\pm1.01$
Neosartoya fischeri Bodhi004	$15.33^{c}\pm4.07$	$37.77^{c}\pm1.97$
Neosartoya fischeri	$16.77^{c}\pm4.44$	$36.40^{c}\pm2.46$
Iprodione (positive control +)	$0.53^{a}\pm1.11$	$52.70^{a}\pm2.59$
Distilled water (negative control -)	$53.17^{d}\pm10.97$	-
F-test	**	**
$LSD_{0.05}$	2.60	4.09
CV (%)	8.14	5.64

Disease incidence and disease reduction was observed at 7 days after inoculation.

*Mean values along with their standard deviations (±) are given in the table. Means within a column followed by different letters are significantly different when analyzed using the least significant difference (LSD) at $p<0.05$, n = 40. ** = Significantly different at $p<0.01$.

plant diseases in various economic crops depending on the testing method and plant growth conditions, including damping-off disease of cucumber and tomato caused by *Pythium aphanidermatum* (Halo *et al.,* 2019), potato and tomato vascular wilt disease caused by *Verticillium* spp. and *Fusarium* spp. (Naraghi *et al.,* 2012; Bahramian *et al.,* 2016), brown spot and dirty panicle diseases caused by rice pathogens (Dethoup *et al.,* 2018a, b) and Chinese kale black spot disease caused by *A. brassicicola* (Komhorm *et al.,* 2021). However, the efficacy of *T. favus* Bodhi003 which caused low suppression of black spot disease was tested under field conditions. The reduction in black spot incidence and disease by *T. favus* Bodhi003 was higher than that by the antagonistic fungi *T. favus* Bodhi001, *T. trachyspermus* Bodhi002 and *N. fischeri* Bodhi004, in agreement with what we found in our previous tests under greenhouse conditions (Komhorm *et al.,* 2021). Our results also revealed that *T. favus* Bodhi003 had the lowest antagonistic activity against *A. brassicicola*, compared with other strains tested both under greenhouse and field conditions in this study. Our study confirmed the efficacy of the *T. favus* Bodhi001 isolate against *A. brassicicola*. A previous study by *Punyanobpharat et al.* (2018) showed the best effective control of *A. brassicicola* under in vitro conditions by the *Neosartoya spinosa* CHA09-A01 isolate. Similarly, *Trichoderma virens* completely inhibited the mycelial growth of *A. brassicicola* causing Chinese kale black spot disease (Intana *et al.,* 2005).



(A) Treated with distilled water. (B) Treated with iprodione 50% WP (1.5 g/L). (C) Treated with a spore suspension of *Talaromyces flavus* Bodhi001. (D) Treated with a spore suspension of *Talaromyces trachyspermus* Bodhi002. (E) Treated with a spore suspension of *Talaromyces flavus* Bodhi003. (F) Treated with a spore suspension of *Neosartorya fischeri* Bodhi004.

Fig 1: Effect of a spore suspension (10^6 spores mL^{-1}) of four antagonistic fungi against *Alternaria brassicicola* under field conditions after spraying Chinese kale plants indicated at 7 days after *A. brassicicola* inoculation.

The field experiment indicated that the leaf sprays in all the treatments with the tested antagonistic fungi on Chinese kale plants resulted in a significant decrease in infection of *A. brassicicola* by reducing the disease incidence compared with the unprotected control. Based on our results, *T. favus* Bodhi001 is the most effective in controlling black spot disease in Chinese kale, by more than 80% in field trials. *T. favus* Bodhi001 is a promising biological control agent (BCA) in controlling *A. brassicicola* causing Chinese kale black spot disease. Although the synthetic fungicide iprodione had the greatest effect on disease incidence and disease reduction, antagonistic fungi are safer and non-toxic for humans and animals and have fewer negative effects on the ecology and the environment than synthetic fungicides. Also, the antagonistic fungus *T. favus* Bodhi001 provides an efficient alternative to synthetic fungicides because *A. brassicicola* is less likely to develop resistance against this antagonistic fungus, which has multiple modes of action. Moreover, antagonistic fungi will be simpler for farmers to apply in the field. These findings will support the use of the antagonistic activity of *T. favus* Bodhi001 for controlling black spot disease in Chinese kale caused by *A. brassicicola* under field conditions.

CONCLUSION

Our results demonstrated that *T. favus* Bodhi001 has potent antagonistic activity against *A. brassicicola* causing Chinese kale black spot disease under field conditions. Its great potential for disease suppression makes the antagonistic fungus *T. favus* Bodhi001 an attractive candidate BCA for controlling Chinese kale black spot disease caused by *A. brassicicola*. It could be useful in the management of black spot disease on vegetable crops and a promising alternative method for reducing the use of synthetic fungicides under field conditions. However, further study is required to evaluate the stability of its control efficacy in comparison with the available commercial fungicide.

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Conflict of interest: None.

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